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Lymphoid Tumors in Mice Receiving Steroid Hormones*

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Estrogenic hormones were first associated with carcinogenesis of the genital tissues because of their capacity to induce growth and sometimes differentiation and secretory activity of these organs or glands (23, 44). Experimentation has revealed that malignant growths of the mammary glands and uterine cervix may follow the prolonged administration of estrogenic hormones. Influences other than endocrine, however, are of etiological significance in mammary carcinogenesis in mice at least (5).

The lymphoid tissues enjoy a peculiar lability among the tissues of the body (13, 57, 61). Humoral factors may affect directly or indirectly the thymus and lymph nodes (13) and it is, therefore, not too unexpected that the incidence of lymphatic leukemia and/or lymphosarcoma has been higher among the groups of estrogen-treated mice than among untreated controls of similar origin. Comparatively few investigators have reported the incidence of lymphomatosis. In 1937 two papers appeared in which it was stated that lymphoid tumors appeared in mice that had received estrogens (22, 39). Since then the appearance of lymphoid tumors among estrogen-treated animals has been mentioned several times (Table I) and three papers have dealt entirely with this problem (25, 27, 39). Because either striking or constant sex differences are not evident in the incidence of lymphomatosis in mice little attention has been given to the etiological role of steroid hormones in experimental lymphatic malignant diseases. The incidence of lymphoid tumors among a large group of estrogen-treated and control mice of several different strains will be reported here.

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** Fellow of The International Cancer Research Foundation.

MATERIALS AND METHODS

Mice from 7 different inbred strains were used.[1] Both the control and treated mice were kept in an air-conditioned room and fed a commercial diet (fox chow) and water. The control mice were used as breeding animals in most instances, their progeny numbering largely among the steroid-treated groups. Ovariectomized and virgin female mice of the C3H strain also served as controls (Table II).

The treated mice received either [2] estradiol benzoate, estradiol dipropionate, estrone, estradiol, equilin benzoate, equilenin benzoate, stilbestrol, triphenylethylene, 9,10-dihydro-9,10-di-*n*-propyl-9,10-dihydroxy-1,2,5,6-dibenzanthracene, or testosterone propionate in solution in sesame oil, or pellets of estrone, stilbestrol, or estradiol dipropionate, or combinations of one or more of the steroid hormones above (Table III). All injections and implantations of pellets were made subcutaneously. The injections of the oily solutions were repeated once or twice weekly, or once every 2 or 3 weeks for periods ranging from 10 weeks to the life span of the animals. Most animals received no more than 0.05 cc. of the oily solutions of the hormone at each injection. Only 1

[1] The mice from the A, C3H, CBA, C12I, JK (59), and C57 strains were obtained from Dr. L. C. Strong or raised from animals obtained from his colony. The original pair of "PM" mice were obtained from Dr. H. B. Andervont who had received them from Drs. Pybus and Miller. The offspring from the original pair were bred brother to sister in this laboratory. Experiments undertaken in collaboration with Dr. J. van Heuverswyn made available 132 of the estrogen-treated mice of the C3H strain.

[2] The estradiol benzoate and testosterone propionate were supplied by Dr. E. Schwenk of the Schering Corporation; estrone, estradiol dipropionate, and estradiol were supplied by Mr. R. C. Mautner of Ciba Pharmaceutical Products, and E. R. Squibb and Sons Biological Laboratory supplied the triphenylethylene and stilbestrol. The pellets were made in the laboratory in a press designed for this purpose. Equilin benzoate and equilenin benzoate were obtained from Dr. A. Girard of Paris.

TABLE I: TABULAR SUMMARY OF INVESTIGATIONS ON LYMPHOID TUMORS AMONG ESTROGEN-TREATED MICE AND THEIR CONTROLS

Investigators	Strain or strains of mice used	Number of mice used	Number or percent of lymphoid tumors	Treatment	Number of controls	Number or percent of lymphoid tumors in controls	Treatment of controls
Gardner (22)	A, C3H, CBA	111	4	Large doses of estrogen	—	None mentioned	—
Lacassagne (39, 40)	R3, 30, 39, 17	?	14	Estrogen or estrogen and pituitary extract	—	None seen during 5 years	
Gardner, Kirschbaum, and Strong (27)	C3H	136	22	Large doses of estrogen	191	1	No treatment, non-estrogen or very small doses of estrogen
Shimkin, Grady, and Andervont (58)	C	61	7	Stilbestrol-cholesterol pellets in mice for 5 to 11 months	143	None in mice 8-16 months old 15 in mice 24 months old or more	
Bischoff et al. (2, 3)	Marsh-Buffalo	40-T* 80-E†	28-36% 30-50%	2,100 r. u. per month 3.3-4.2 mgm. total dose	40 40	18% 10%	Sesame oil Sesame oil
Bischoff et al. (4)	Marsh-Buffalo	36 36	34% 8%	"Toxic amounts" "Toxic amounts"	43	5%	Sesame oil
Gardner (25)	C3H, CBA	303	76	Large doses of several different estrogens	481	9	No treatment, non-estrogen treatment, or sesame oil

* T—theelin.
† E—estradiol.

TABLE II: INCIDENCE OF LYMPHOID TUMORS AMONG UNTREATED MICE OF SEVEN INBRED STRAINS

Strain	Sex	Number	Number with leukemia	Age, days	Number without leukemia	Average age, days
C3H	♀	210	1	856	209	324
	♂	91	1	453	90	479
	V♀	111	2	634	109	550
	C♀	50	—	—	50	530
	C♀	19	1	560	18	670
CBA	♀	19	—	—	19	672
	♂	43	2	490	41	545
PM	♀	38	—	—	38	500
	♂	20	—	—	20	565
A	♀	57	—	—	57	414
	♂	25	—	—	25	527
C57	♀	43	3	522	40	559
	♂	16	—	—	156	616
JK	♀	26	—	—	26	428
	♂	11	1	679	10	400
C12I	♀	32	—	—	15	428
	♂	11	—	—	2	490
Totals		822	11		811	

V—virgin females.
C—castrated females.

pellet of hormone was implanted. The injections or implantations were begun when the mice ranged from 22 to 141 days of age and most frequently when they were from 25 to 45 days old. Pellets of the different estrogens ranged from less than 1 mgm. to 8 mgm. They were implanted by forcing them through a 13 gauge hypodermic needle by means of a wire plunger.

The mice were examined daily throughout the course of the experiments. When death occurred, or an animal was killed because death was considered imminent, autopsies were performed and all tissues and organs were observed. If the lymph nodes, spleen, or thymus were enlarged the tissues usually were fixed either in Bouin's fluid, 10 per cent formaldehyde, or Helly's fluid, sectioned, and stained with hematoxylin and eosin. If the tissues were not fixed air-dried imprints were prepared and stained with May-Grünwald-Giemsa stain. Air-dried imprints of the spleen, thymus, and bone marrow when it could be obtained, and of the lymph nodes or tumorous nodules were usually prepared even when the fixed and sectioned tissues were also studied. If lymphomas were not present the tissues were not always saved. The evidence upon which the following studies are based, therefore, is the histological and the cytological confirmation of malignant growth suspected from gross observations at autopsy. In addition a number of the tumors have been transplanted into other mice of the same strain and the autonomy of the neoplasia established.

The animals have been studied during the last 5 to 6 years. Not all of them were living at any one period, nor did all groups represent the same generation of inbreeding.

Incidence of Lymphoid Tumors among the Untreated Control Mice

Only 11 mice with lymphoid tumors were found among the 822 uninjected control animals observed (Table II). The highest incidence of spontaneous tumors occurred in the C57 black strain, in which 3 of the 59 mice died with lymphoid neoplasia. Spontaneous lymphoid tumors were not observed in mice of the C12I, A, or PM strains. The number of observations, however, is inadequate to permit the conclusion that lymphoid tumors do not appear among mice of these 3 strains. The extremely low incidence of lymphomatosis in all strains, with the possible exception of the C57, indicates that they cannot be considered as particularly susceptible to the acquisition of such tumors.

The lymphoid tumors appeared in aged mice in most instances, usually in animals beyond the average age of the group as a whole (Table II). From the data available no indication of any sex difference in the incidence of the lymphoid tumors was evident.

Table III: Number of Lymphoid Tumors among Control and Estrogen- or Steroid-Treated Mice of 7 Different Strains

	Controls		Estrogen		Estrogen and testosterone		Testosterone	
Strain	Number	Tumors	Number	Tumors	Number	Tumors	Number	Tumors
C3H	481	5	747	109	182	6	41	0
CBA	62	2	445	67	39	0	12	1
PM	58	0	143	22	24	3	—	—
A	82	0	94	3	41	0	—	—
C57	59	3	170	3	30	0	—	—
JK	37	1	64	3	26	0	—	—
C12I	43	0	136	8	36	0	12	0
Totals	822	11	1,799	215	378	9	65	1
Percentage of total with leukemia		1.34		11.9		2.34		1.54

The data available failed to reveal any sex difference in the incidence of spontaneous lymphomatosis. Ovariectomized female mice of the C3H strain did not acquire lymphomas more frequently than virgin females of the same strain. The apparent lower incidence of lymphomas among the breeding females of this strain might be attributed to the high incidence of mammary tumors in comparatively young mice.

Generalized infiltrations of the lymph nodes, spleen, liver, and other tissues were found in most of the untreated animals in which lymphomas developed. None of the control mice that died with lymphoid tumors showed mediastinal extensions involving the thymus. The only extensive mediastinal metastases were found in parathymic nodes of two leukemic mice of the C3H strain and in one animal each of the CBA and C57 strains.

In addition to those just mentioned, a few mice of the C3H strain had very large hyperemic and edematous mesenteric lymph nodes. Two attempts to transplant such lesions were unsuccessful. This apparent lack of autonomy together with the histological char-

acteristics of these lesions gave adequate evidence of their benign nature and they were therefore not included among the animals with lymphoid neoplasia. Similar lymphoid conditions were found occasionally among some of the estrogen-treated mice, especially those on the lower doses.

A number of mice, especially of the C57 strain, showed extensive myeloid metaplasia. The spleens and livers as well as some of the lymph nodes of these animals were greatly enlarged and contained cells of both the erythroid and leucocytic series at all stages of development. Careful study was made to prevent the inclusion of such animals among those with lymphomas.

Incidence of Lymphoid Tumors among Estrogen-Treated Mice of Several Different Strains

It is impossible to consider the data solely from the standpoint of the strain of mice used and the number or percentage of lymphomas observed. The mice of some strains survived intensive treatment with estrogens very poorly, few living to an age at which lymphomas might be expected. When all the data on estrogen-treated mice were combined into groups according to the strains of mice the incidence among the strains ranged from 15.4 per cent to 1.8 per cent (Tables III and IV). The incidence was 14.4, 15.1,

Table IV: Percentage of Mice of Different Inbred Strains That Had Lymphoid Tumors at Death

Strain	Control	Estrogen-treated	Estrogen- and testosterone-treated	Testosterone-treated
C3H	1.0	14.4	3.3	0
CBA	3.2	15.1	0	8.3
PM	0	15.4	12.5	—
A	0	2.1	0	—
C57	5.0	1.8	0	—
JK	2.7	4.7	—	0
C12I	0	5.9	0	0

and 15.4 per cent for the C3H, CBA and PM strains respectively, 4.7 and 5.9 per cent for the JK and the C12I strains, and 2.1 and 1.8 per cent for the A and C57 strains. The mice might be divided into 3 groups according to the incidence of lymphoid neoplasia, the estrogen-treated mice of the first three strains mentioned above being highest, the JK and C12I strains intermediate, and the A and C57 lowest. In all except the C57 strain the incidence was higher among the estrogen-treated than among the control groups. From the data available the incidence of tumors among the control mice could not be correlated with the incidence among the estrogen-treated animals. Such a correlation is of limited merit, however, since all the strains, with the possible exception of the C57, showed such a low incidence of spontaneous lymphomatosis.

Influence of Sex upon the Incidence of Lymphoid Tumors in Estrogen-Treated Mice

The incidence of lymphoid tumors and the number of mice observed were sufficiently large in only 4 strains to merit a separate classification of the data on the basis of sex (Table V.) The relative number of lymphomas was approximately twice as high among the female CBA and C3H mice as among the males of these two strains. Because males and females from the same litters were used, this difference cannot be attributed to any possibility of selection within the

Table V: Incidence of Lymphoid Tumors among Estrogen-Treated Male and Female Mice

	Males		Females	
Strain	Number	With lymphoid tumors, per cent	Number	With lymphoid tumors, per cent
C3H	539	12.6	188	20.7
CBA	175	13.6	144	25.0
PM	87	17.3	56	12.5
C12I	73	6.8	63	4.7

strain. Also the number of mice of the two sexes represented in the groups treated with the different doses of estrogen were relatively comparable. Among the PM mice, however, the incidence of tumors was higher among the males. The tumors that did appear among the females of this strain were observed at a significantly greater average age (Table VI). The incidence of lymphoid tumors among the C12I mice was also greater among the males than the females. Although only 3 lymphoid tumors appeared among the mice of each of the other three strains, both males and females from each strain were affected.

Influence of the Amount of Estrogen Administered on the Incidence of Lymphomatosis

An arbitrary division of the doses of hormone was made as indicated in Table VI. The groups were arranged on the basis of effective doses rather than the amount of hormone administered or expressed gravimetrically. For example, a pellet of estrone from which a few micrograms are absorbed each week is relatively much more toxic (effective) than the weekly injection of 50 μgm. of the chemical injected in solution in oil. Such pellets weighing from 2 to 3 mgm. persisted in the subcutaneous tissues throughout the life of their bearers, although their weights were reduced. The relative toxicity or effectiveness of the different estrogens or of the same estrogen variously administered was estimated by noting the relative survival of the treated animals, capacity to gain in weight, and evidence for excessive stimulation of the genital tissues. Pellets of estradiol or stilbestrol were much

more soluble than the pellets of estrone, persisting in the subcutaneous tissues for only 3 months, and were extremely effective or toxic while present.

strain survived the high doses poorly, the average age attained being only about 175 and 130 days respectively.

The mice of the C3H and CBA strains that received

TABLE VI: INFLUENCE OF AGE UPON INCIDENCE OF LYMPHOID TUMORS

Strain	Designation of group *	Males		Females	
		Average age, days	With leukemia, average age, days	Average age, days	With leukemia, average age, days
C3H	Total	368 (471)†	331.6 (68)	311 (148)	345 (39)
"	L	271 (211)	276 (33)	231 (42)	267 (22)
"	LI	436 (70)	355 (30)	257 (58)	369 (14)
"	S	452 (190)	427 (5)	509 (48)	561 (3)
CBA	Total	401 (151)	350 (24)	319 (108)	325 (36)
"	L	267 (77)	249 (12)	246 (61)	290 (24)
"	LI	450 (27)	326 (6)	369 (16)	283 (5)
"	S	591 (47)	509 (6)	435 (31)	601 (7)
PM	Total	178 (72)	238 (15)	167 (49)	318 (7)
"	L	188 (49)	234 (9)	168 (36)	308 (5)
"	LI	129 (10)	193 (1)	91 (8)	—
"	S	293 (13)	256 (5)	285 (5)	466 (2)

* L—large doses—weekly injections of 10, 25, or 50 μgm. of estradiol dipropionate, 16.6, 25, 33.3, or 50 μgm. of estradiol benzoate or pellets of estrone. LI—large doses interrupted—pellets of estradiol, estradiol benzoate, or stilbestrol which were completely absorbed in less than 3 months or injections of high doses of estradiol benzoate or dipropionate for 10 to 12 weeks. S—small doses—weekly injections of 1, 8.3, or 10 μgm. of estradiol benzoate, 50 μgm. of estrone, 25 to 250 μgm. of stilbestrol, 5 mgm. of triphenylethylene, or 1.25 mgm. of 9,10-dihydro-9,10-di-*n*-propyl-9,10-dihydroxy-1,2,5,6-dibenzanthracene.

† Figures in parentheses refer to the number of animals in each group.

The mice receiving the largest doses survived a much shorter time than those given the lowest doses (Table VI). With the exception of the PM and JK

TABLE VII: INFLUENCE OF AMOUNT OF ESTROGEN AND OF DURATION OF ADMINISTRATION UPON INCIDENCE OF LYMPHOID TUMORS IN MICE

Strain	Dose *	Number of mice	Number of tumors	Per cent with tumors
C3H	L	308	55	17.9
	LI	172	44	25.6
	S	267	9	3.4
CBA	L	222	41	19.9
	LI	54	11	20.6
	S	159	15	9.5
PM	L	99	14	14.1
	LI	19	1	5.3
	S	25	7	28.0
A	L	54	2	3.7
	S	40	1	2.5
C57	L	101	2	2.0
	S	69	1	1.45
JK	L	26	0	0
	S	38	3	7.9
C121	L	75	6	8.0
	S	61	2	3.3

* See footnote on Table VI.

strains the incidence of lymphoid tumors was uniformly higher in the mice receiving the larger doses (Table VII). The mice of the PM group and the JK

large doses of estrogen for 10 weeks to 3 months and then had the treatment discontinued acquired relatively more lymphoid tumors than the mice continuously treated (Table VII). They survived, however, a significantly longer time than did the animals treated continuously (Table VI).

The incidence of lymphoid tumors can be increased from approximately 1 per cent to 20 or 25 per cent by subjecting the animals of the C3H and CBA strains to large amounts of estrogens for prolonged periods or for periods of 70 days or more. Those animals in which treatment was stopped after 10 weeks to 3 months revealed lymphoid tumors at variable but prolonged intervals after the cessation of treatment, at which time many of them showed no other indication of their previous exposure to estrogens.

THE EFFECT OF THE ESTROGENIC CHEMICAL USED UPON THE INCIDENCE OF LYMPHOID TUMORS

It is difficult to consider the action of the different estrogenic chemicals solely on a qualitative basis because of their extreme ranges of physiological activity. However, an adequate number of mice of the C3H strain were subjected to treatment with enough of the different estrogenic substances to permit the conclusion that when any one of the estrogens was given in adequate amounts lymphoid tumors developed at

an increased incidence (Table VIII). The greater the physiological activity, *i.e.*, the number of mouse units per mgm., of the estrogen the more effective a given dose in the induction of lymphoid tumors. Pellets of estrone, estradiol, and stilbestrol were all very effective although the pellets of the latter two chemicals persisted in the subcutaneous tissues for only 2 to 3 months. The number of tumors among the mice that received the estradiol dipropionate was greater than among those receiving estradiol benzoate. The former compound, when given in amounts much in excess of a minimal effective dose, was much more active per unit of weight than the latter in producing a prolonged and intense estrogenic reaction.

Table VIII: Number of Lymphoid Tumors among Mice of the C3H Strain That Had Received One of Several Different Estrogenic Chemicals *

Estrogenic chemical	Dose	Groups on basis of dose †	Number of mice treated	Number of lymphoid tumors	Per cent with lymphoid tumors
Estrone	50 μgm. weekly	L	30	0	0
Estrone	Pellets	L	81	15	19
Estradiol	"	LI	12	4	33
Estradiol dipropionate	50 μgm. weekly	L	78	18	23
" "	Pellets	LI	55	16	29
" "	25 μgm. weekly	L	18	4	22
" "	10 μgm. "	L	30	4	13
" "	10 μgm. " × 10	LI	54	10	19
Estradiol benzoate	50 μgm. "	L	18	2	11
" "	25 μgm. "	L	35	5	14
" "	10 μgm. "	S	18	1	6
Stilbestrol	250 μgm. "	S	24	0	0
"	25 μgm. "	S	18	0	0
"	Pellets	LI	51	13	26
Equilin benzoate	0.1 mgm. weekly	L	12	4	33
Equilenin benzoate	0.1 mgm. "	S	12	0	0
9,10-Dihydro-9,10-di-*n*-propyl-9,10-dihydroxy-1,2,5,6-dibenzanthracene	0.125 mgm. "	S	18	2	11
Triphenylethylene	5 mgm. "	S	20	1	5

* A number of mice of the C3H strain that received smaller doses of estrogen or combinations of 2 different estrogens are not included.

† See Table VI.

The Effect of Testosterone on the Incidence of Lymphoid Tumors

Of the 65 mice that received weekly subcutaneous injections of 0.625 to 2.5 mgm. of testosterone propionate one had at death a lymphoid tumor (Table IX). The incidence was approximately the same as in the untreated control mice. The mice of this group survived treatment longer than the estrogen-treated mice and lived approximately as long as the untreated controls. Testosterone propionate had neither an inhibitory nor a stimulating effect on the production of lymphomas.

Table IX: Incidence of Lymphoid Tumors among Mice Given Testosterone Propionate

Strain	Number of mice	Number of lymphoid tumors	Average age at death, days	Mice with tumors, average age at death, days
C3H	41	0	477	—
CBA	12	1	743	816
C12I	12	0	484	—

The Effect of Testosterone Propionate Plus Estrogens upon the Incidence of Lymphoid Tumors

Nine lymphoid tumors (2.3 per cent) were found among the 378 mice that received subcutaneous injections of 8.3 μgm., 16.6 μgm., 33.3 μgm., 50 μgm. of estradiol benzoate or pellets of estrone with 0.625, 1.0, 1.25, or 2.5 mgm. of testosterone propionate (Table X).

Table X: Incidence of Lymphoid Tumors in Mice Given Testosterone Propionate in Combination with Estrogen

Strain	Number of mice	Number of tumors	Average age at death, days	Mice with tumors, average age at death, days
C3H	182	6	375	397
CBA	39	—	465	—
PM	24	3	343	320
A	41	—	401	—
C57	30	—	363	—
JK	26	—	268	—
C12I	36	—	414	—
Total	378	9		

The incidence of lymphoid tumors slightly exceeded that observed in the control animals.

The relative number of lymphoid tumors in this group was largest in the PM strain. The average age at death of the animals of the PM group was 343 days or over 100 days more than among the estrogen-treated mice. The only other strain in which lymphoid tumors appeared under such treatment was the C3H. Slightly over 3 per cent of these mice died with lymphoid tumors at an average age of 397 days.

The injection of testosterone propionate in combination with estrogen reduced the number of lymphoid tumors that would have been expected if the estrogen had been injected alone.

Gross and Microscopic Characteristics of the Lymphoid Tumors in Estrogen-Treated Mice

Most of the estrogen-treated mice that acquired leukemia became dyspneic because of involvement of organs in the mediastinum. The thymus, parathymic nodes, lungs, heart, trachea, and the walls of the great blood vessels were infiltrated massively in nearly all the animals (approximately 80 per cent) that developed leukemia following estrogen treatment (Fig. 1). The normal histological structure of the thymus was no longer evident, the organ being replaced almost entirely by large masses of lymphoid cells. The lymphoid cells invaded the heart directly, passing in and about the muscle fibers and penetrating to the endocardium. The lungs were infiltrated at the hilus and along the bronchi (Figs. 2 and 3). The lung structure in the regions of extensions or metastases was obliterated. The walls of the trachea and blood vessels were infiltrated and the serosa was almost entirely replaced by lymphoid cells.

In the majority of the animals organs other than those of the mediastinum were involved. The spleen was enlarged in many of these mice but in others it contained leukemic cells without being larger than normal (Fig. 4). The malpighian corpuscles were very indefinite in the enlarged spleen and usually little differentiation between red and white pulp was observed, since the hyperplasia of the follicles was so great as to obscure intermediate tissue. Many mitoses were observed in the leukemic cells.

Although all the lymph nodes from each of the lymphomatous animals were not examined microscopically, those of many of the animals with lymphomas were involved. The mediastinal nodes were affected most frequently but the abdominal and the subcutaneous nodes were involved also in many of the animals. The lymphoid cells were hyperplastic, obliterated the peritrabecular and subcapsular sinuses, and often extended beyond the capsule.

The livers of many of the leukemic animals were enlarged and pale red in color. The hepatic invasion by lymphoid cells was usually in the periportal areas but sinusoidal invasion also was found (Fig. 5). The livers of several of the mice with leukemia as well as those of other mice in which leukemia did not appear showed large abscesses and occasionally cystic spaces. The intrahepatic abscesses were largely in mice of the A strain.

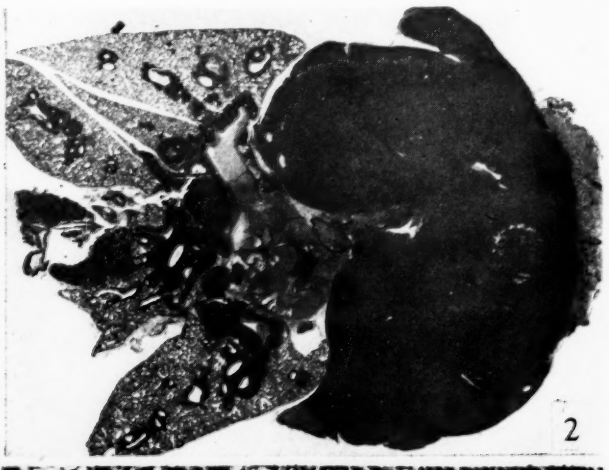
The kidneys, when extensively infiltrated, were enlarged and pale in color. Leukemic infiltrations were found in the interstitial tissue and often invaded the cortex to such an extent that large nodules of leukemic cells were formed (Fig. 6). The subcapsular regions in many animals were invaded.

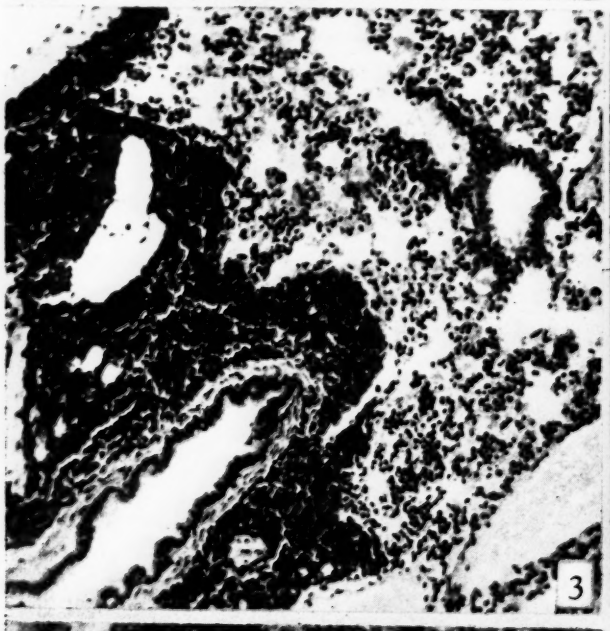
Other organs such as the ovary (Fig. 7), uterine tubes, and uterus also were involved in the leukemic process.

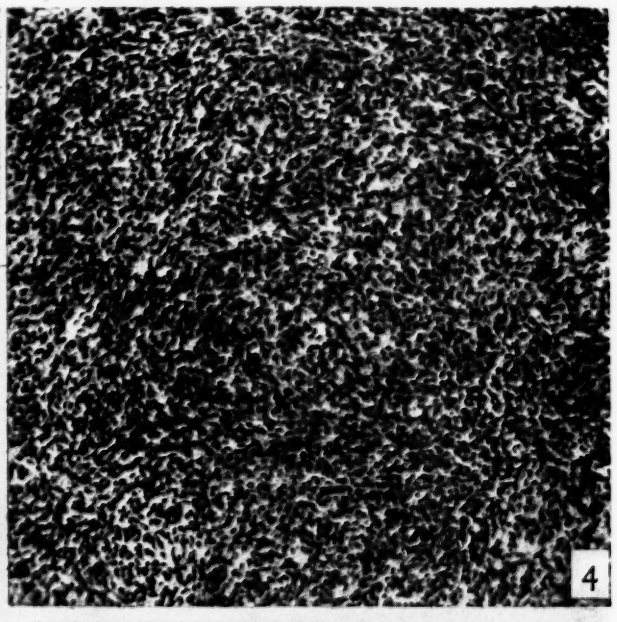
Cytological studies of the leukemic cells were carried out on air-dried imprints stained with May-Grünwald-Giemsa. The leukemias were all of the lymphatic type. There was no variation in cell type in the different areas of infiltration or metastasis. In general lymphomas composed of two types of cells could be distinguished. Some were composed of cells that were very large and often contained indented nuclei with several indefinite nucleoli (Fig. 8). There was a distinct difference between chromatin and parachromatin in the nuclei of these cells. They had varying amounts of cytoplasm, which was peripherally quite basophilic and which blended into an inner hyaloplasmic zone. These malignant cells resembled reticular cells. Tumors composed of cells of this type did not seem to show a tendency to metastasize but usually remained confined to the mediastinum, where they appeared to arise from the thymus. One tumor of this type (6C3HED) was carried through 25 generations and never showed true metastases although it was an extremely rapidly growing tumor. In such transplants there was no dependency on estrogen for continued growth of the cells. Other tumors metastasized and showed a high incidence of blood involvement, as has been described previously (42).

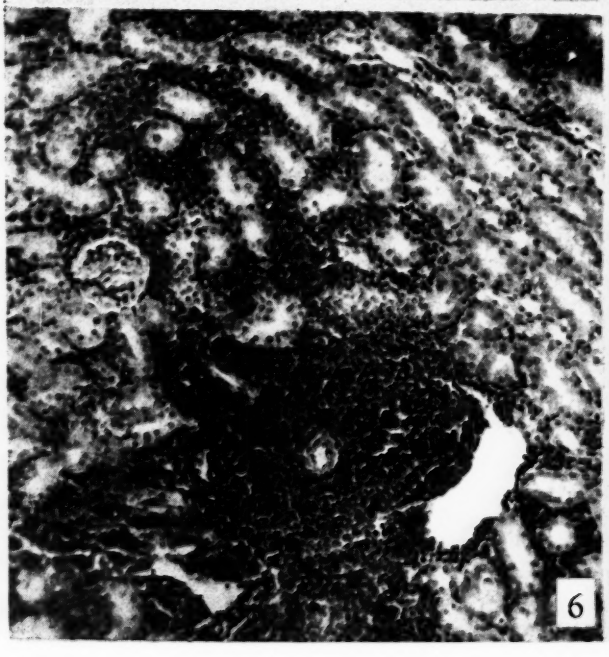
Most of the tumors were composed of cells smaller than those described above (Fig. 9). The nuclei of these cells varied from very undifferentiated myeloblastic types with finely stippled chromatin to cells that contained nuclei having larger amounts of darkly staining chromatin arranged in large band-like formations. The maturation of the younger cells to more or less mature lymphocytes of a normal type was evident. Cells of this type metastasized most frequently; their hosts usually showed generalized lymphomatosis.

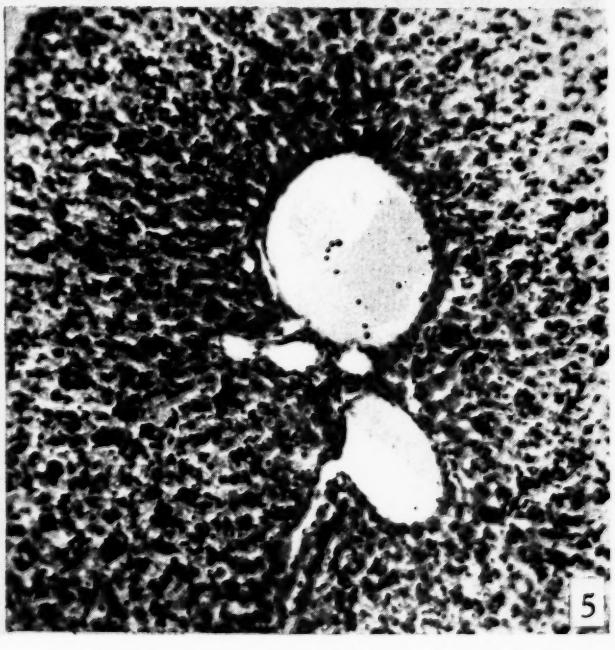
Although blood smears were not obtained from many of the lymphomatous animals several mice from





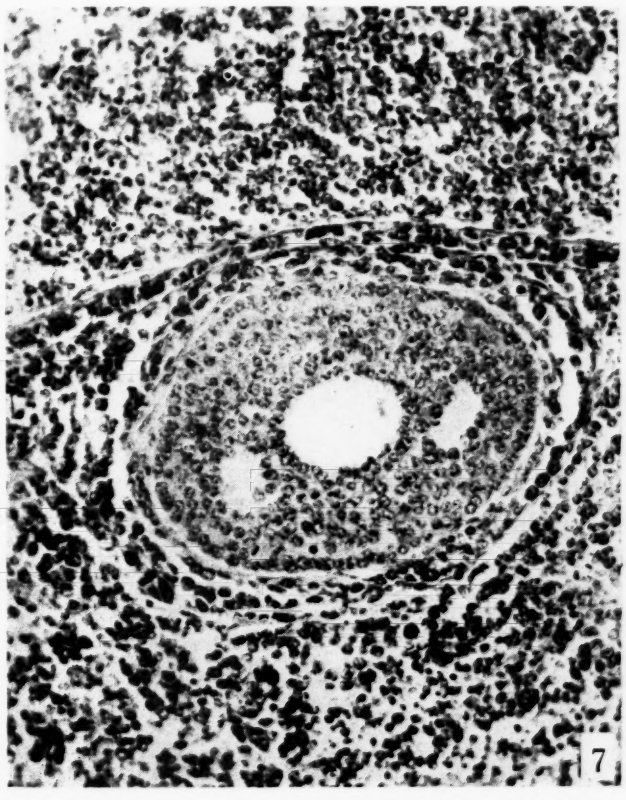






FIGS. 1-6

which smears were available showed definite leukemic pictures. In such cases the cells were more or less having sieve-like nuclei and nucleoli were noted in the blood but these cells were observed rarely. The dis-



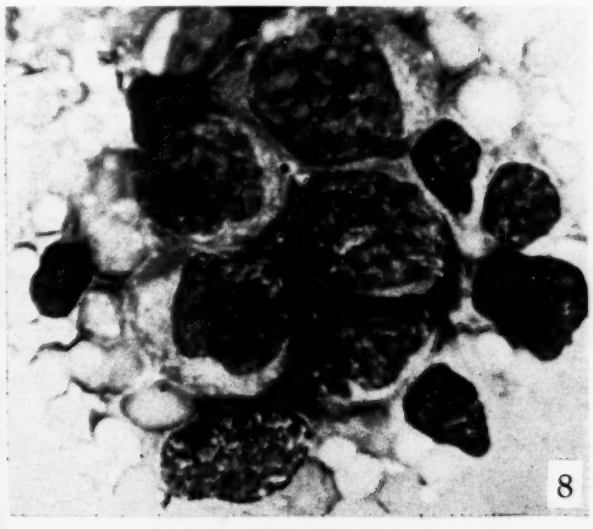


FIG. 7.—Lymphomatous infiltrations of the ovary. Ovarian tissue completely replaced by leukemic cells with the exception of the follicle. × 200.

FIG. 8.—Lymphomatous cells of a large type (from 6C3HED—a mouse of the C3H strain that had a pellet of estradiol implanted). Note multiple nucleoli and sharp contrast between nuclear chromatin and parachromatin. The cells resembled reticular cells. Cells of this type were usually not highly invasive. This tumor had not metastasized in any of the 25 generations. × 1,800.

FIG. 9.—"Malignant lymphocyte" from the blood of a lymphomatous animal. The hyperchromatic nucleus and the dark basophilic cytoplasm are characteristic of leukemic lymphocytes in mice. × 1,500.

mature lymphocytes and did not possess characteristics that might be called leukemic other than that they had hyperchromatic nuclei and deeply basophilic cytoplasm (Fig. 9). Such cells have been described as malignant lymphocytes (35). A few younger cells

tinction between normal and leukemic lymphocytes in mouse blood was not determined easily.

Many of the animals that had been treated with estrogens for a long period of time but were not considered to have lymphomatosis had spleens that were

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Viscera of 3 estrogen-treated mice with lymphomas. Large mediastinal (thymic) tumors; enlarged spleens and pale livers. Two are of the C3H strain and 1 is of the CBA strain. Animal at left had received 1.15 mgm. estradiol dipropionate in 166 days (23 weeks), starting at 44 days of age. At death the white blood cell count was 39,800. Animal at right (C3H mouse) received 1.85 mgm. of estradiol dipropionate in 265 days, starting at 37 days of age. The white blood count was 3,800. This tumor was successfully transplanted into 2 mice of same strain. Mouse in center (CBA strain) received 0.8 mgm. of estradiol benzoate in 229 days, starting at 40 days of age. The white blood count was 30,000. × ½.

FIG. 2.—Cross section of the thoracic contents of mouse of the C3H strain that had received 0.75 mgm. of estradiol benzoate in 212 days, starting at the age of 53 days. The large tumor developed in the anterior mediastinum and extended throughout the mediastinum and along the bronchi. This mouse showed generalized involvement of all the tissues. × 4.

FIG. 3.—Invasion and destruction of lung tissue and peribronchial invasion of lymphocytic cells. × 100.

FIG. 4.—Spleen from an estrogen-treated hybrid mouse with a lymphoid tumor. The hyperplasia of reticular cells resembled the condition found in many of the estrogen-treated animals, of which many were not considered lymphomatous since the lesions were not invasive. × 100.

FIG. 5.—Liver of estrogen-treated CBA mouse showing a diffuse sinusoidal infiltration. In other mice periportal invasion was found. × 100.

FIG. 6.—Kidney of estrogen-treated mouse of the PM strain. The infiltrations were usually in the interstitial tissue but were sometimes subcapsular. × 100.

definitely atypical. Not all the organs were available for study in these animals and, therefore, whether the changes described frequently occurred in the thymus and nodes could not be determined. Definite hyperplasia of reticular cells in the spleens appeared in many of the treated animals. The malpighian corpuscles were enlarged and contained cells with large nuclei and not so densely packed as the lymphocytes making up the greater part of the normal follicles. The hyperplasia resulted in an increased extension of reticular cells along the vessel walls, especially in the perimalpighian region. Hyperplasia of the splenic reticular tissue frequently was found in mice that were definitely not leukemic. Estrogens given over prolonged periods brought about hyperplasia of the reticuloendothelial cells in the spleen. Whether such changes were related to the subsequently developing leukemia was not determined.

Several of the estrogen-induced lymphomas have been transplanted and have grown in hosts from the same strain. The cells were not dependent on estrogen for their continued growth.

The Relation of the Tendency for the Development of Lymphoid Tumors to the Tendency to Acquire Other Types of Tumors

It was stated previously that the incidence of lymphoid tumors among the estrogen-treated mice was highest for the mice of the C3H, CBA, and PM strains and lowest for the mice of the A and C57 strains. The tendency for mammary (59), testicular (6, 21, 26, 33), and hypophyseal (30) tumors to appear among the mice of these different strains also varied (Table XI). These tendencies could not be associated in any way with one another.

Table XI: Relative Incidence of Tumors among Estrogen-Treated Mice

Strain	Lymphoid	Mammary	Testicular	Pituitary
C3H	+++	++++	—	—
CBA	+++	+++	—	—
PM	+++	—	—	+
C12I	+	++	—	—
JK	+	—	+	—
A	—	+++	+++	—
C57	—	—	—	+++

When large amounts of estrogen were given, however, the incidence of mammary tumors was much lower than when smaller amounts were given. The amount of mammary growth was less than that attained in mice receiving smaller amounts of estrogen (24). To this extent within a single strain, such as the C3H, there was an inverse relation between the incidence of lymphoid and mammary tumors among the groups of mice on the different dose levels.

Discussion

The strain-limited appearance of spontaneous leukemia in mice has revealed that genetic factors contribute to the attainment of this type of neoplasia or at least to a predisposition for such neoplasia (35, 45, 53). The experimental induction of lymphomas in mice has for the most part been limited to methods that increased the incidence of the disease in strains or stocks of animals definitely possessing an appreciable or a high genetic susceptibility to the spontaneous occurrence of neoplasias of lymphoid or myeloid elements. The technic used most frequently has been the protracted percutaneous or parenteral application of several of the carcinogenic hydrocarbons dissolved in one of several different solvents to mice of relatively "high leukemia" strains. Such procedures have increased the incidence of leukoses and in many instances decreased the age at which these occurred in these strains (7, 15, 16, 31, 41, 47, 48, 49, 51, 52). Painting of mice of the F strain with methylcholanthrene dissolved in benzene resulted in a pronounced precocity of the disease (36, 37). Approximately 38 per cent of the treated mice of this strain showed leukemia at 300 days of age in contrast to a spontaneous incidence of 14 per cent at the same age in controls. Of a smaller number of animals of the same strain treated with benzpyrene over 45 per cent had leukemia at 300 days. The preleukemic latent period was not shortened by painting with benzene. Also, painting with carcinogens had little if any effect on C3H mice that possessed no significant predisposition for spontaneous leukemia. However, it has been reported that the percutaneous application of a carcinogen has caused a significant occurrence of leukemia in one stock of mice that showed a low incidence of the disease spontaneously (18). The protracted painting with methylcholanthrene increased the incidence of leukemia in mice of the Rf stock from a control (spontaneous) level of 2 per cent to more than 30 per cent (11 leukemias in 32 animals).

Roentgen-ray treatment has been used to increase the occurrence of leukosis in mice. Only 19 leukemias appeared in 5,500 roentgen-rayed animals; 3 cases of leukemia were present in 10,500 untreated controls (38). Later other investigators obtained a much more significant increase in leukemia in several stocks of mice subsequent to roentgen irradiation (19). A more striking increase in the incidence of leukemia in several stocks of mice has been accomplished by combining treatment by roentgen rays with the percutaneous application of methylcholanthrene (46). By means of this combined technic the incidence of leukemia in Rf × Ak hybrids (F_1) was increased from 12 per cent in untreated animals to over 75 per cent in treated animals. Subcutaneous injection and implan-

tation of the carcinogenic hydrocarbons have in some instances increased the incidence of leukemia but usually to a lesser degree than prolonged painting with the same substances (9, 16, 18, 32). A considerable number of leukoses have been produced by injection of benzpyrene directly into the spleens of mice (1, 20). Other workers were unable to produce any lymphoid neoplasia by implanting crystals of methylcholanthrene and of estrone directly into lymph nodes of mice from several strains (17). It also has been reported that subcutaneous injections of benzol in olive oil, and of indol, have resulted in the appearance of leukemia in mice (8, 43).

The neoplasias of lymphoid and myeloid tissues that appeared in mice treated by the several methods described above included lymphatic leukemia, myelogenous leukemia, monocytic leukemia, and lymphosarcoma. All these terms, in addition to the more general ones of leukemia, leukosis, and lymphomatosis, have been used by various authors to describe the neoplastic conditions.

Experiments concerned with the instigation of lymphoid tumors in mice have revealed that the carcinogenic hydrocarbons and estrogens are most effective in increasing the incidence of such tumors. The carcinogenic hydrocarbons have been especially effective in increasing the incidence, or reducing the age incidence, of leukoses in mice of most of those strains or groups that showed some tendency to acquire such malignancies. Estrogens, on the other hand, increased the incidence of lymphomatosis among some strains that had a very low incidence of such tumors among the untreated controls, but not in all strains of mice. Estrogens have not been adequately tested in mice from strains showing a high incidence of spontaneous leukosis.

How estrogens may act in the animals to increase the incidence of lymphomas has not been determined. These substances, however, affect the animal organism much more extensively than merely by eliciting growth and secretory activity of some of the genital tissues and inducing the development of the secondary sex characters. Estrogen-treated rats or mice showed an increase in the size of their pituitaries and their adrenal cortices (10, 11, 56). The thymus regressed in estrogen-treated rats. Unlike certain other toxic substances estrogens and some other steroid hormones caused thymic regression even in the absence of the adrenal gland (34, 54). Although attempts were not made to assess the action of estrogens on lymphoid tissue the observations made during the present studies indicated that the amount of lymphoid tissue was decreased until the lymphomas appeared.

More recent experiments have revealed that the thymus and lymph nodes regressed rapidly in animals given adrenal cortical steroids (corticosterone) or adrenocorticotropic hormones (13, 50). The number of circulating lymphocytes also decreased (14). The lymphoid tissues of the estrogen-treated mice might be indirectly affected through the adrenal gland or more possibly affected through the pituitary and adrenal. The fact that adrenalectomized animals receiving estrogens have a smaller thymus than the adrenalectomized controls indicated that estrogens acted independently of the adrenal gland.

The hematopoietic tissues of the mice that received estrogen were altered in one other way. The bone marrow was replaced largely by osseous tissue (28, 29). Some experimental observations have revealed that bone may be formed by a metaplastic transformation of the reticular cells of the bone marrow. These observations merely indicated that estrogens have had in some instances profound effects upon reticular tissues of the bone marrow at least. Dogs given large amounts of estrogen became agranulocytic, anemic, and died (12, 60). Although similar conditions were not observed in the mice the amount of erythropoiesis in their spleens was apparently increased.

The observations reported here have revealed that relatively brief exposures of mice to estrogens was followed by as high an incidence of lymphomatosis as more prolonged treatments. The changes induced during short but intense treatments were irreversible in so far as they were related to the predisposition to lymphomas. The lymphoid tumors appeared in these mice several months after their genital and skeletal tissues had recovered largely from evidence of their previous exposure to estrogens. The lymphoma-inducing activity apparently was attained early in these experiments. This is not necessarily a unique response in respect to either endocrines or cancer. The injection of estrogens into rats less than 5 days of age will induce morphological and functional disturbances that will appear in later life and persist until death (63). The consumption of milk from mothers of tumor-susceptible strains may transmit to genetically suitable animals a tendency to acquire mammary tumors, those growths appearing much later in life (5).

The mice receiving the large interrupted doses of estrogens usually survived longer than those animals treated continuously. Because large amounts of estrogen inhibit mammary growth (24) the incidence of mammary tumors was reduced. Both the facts above might contribute to the high incidence of lymphomas among the mice treated for short periods.

Estrogens and androgens mutually inhibit certain reactions elicited in animals (62). For example, mice that received testosterone propionate in adequate amounts in combination with estrogens did not show the medullary ossification observed in mice that had

received the same amount of estrogen alone (29). It was of interest that the administration of androgen reduced the incidence of lymphomas in mice given estrogen. This observation indicated that the estrogens might not be directly "toxic" as lymphoma-inducing agents but that the tumorous response resulted from some physiological response counteracted by the androgen. On the other hand it was not impossible that androgen opposed a direct effect of estrogen as a lymphoma-inciting agent. The fact that both the steroid and nonsteroid estrogens were effective when given in what approached physiologically comparable amounts also might indicate that these chemically quite different substances act indirectly. So little is known of the mechanism through which estrogens affect the genital tissue that it is probably futile to attempt to explain their mode of action on lymphoid tissues. The lymphoma-inducing activity of the several different strains was, as far as could be determined, correlated directly with the amounts of estrogen administered in terms of effective dose levels.

No peculiar concomitant biological reactions were detected that could be associated with the strain-limited induction of lymphomas among the estrogen-treated mice (Table XI). The bones of mice of all strains were altered by estrogens. The osseous changes were most rapid and intense in mice of C3H, JK, and PM strains and hence could not be correlated with the tendency of mice of these strains to acquire lymphomas. The data available also failed to reveal that the different susceptibilities could be associated with the action of estrogens on any of the other glands or tissues. The hereditary predisposition of the estrogen-treated mice of some strains (C3H, CBA, PM) to acquire lymphomas was as unique as the predisposition of mice from other strains similarly treated to the acquisition of mammary (C3H, A), hypophyseal (C57), or testicular tumors (A, JK).

The lymphomas occurring in both young and old mice were morphologically and clinically similar except that fewer generalized lymphomas were found among those animals in which the disease appeared at less than 200 days of age, whereas nearly all the lymphomatous animals (92 per cent) over 400 days old had metastases. The greatest number of tumors occurred in animals from 200 to 400 days of age, and of these about one-fifth (19 per cent) were entirely localized in the superior mediastinum or thymus and adjacent tissues. The duration of treatment or the dose of estrogen did not alter the incidence of the general or intrathoracic manifestations of the lymphomas except in so far as they influenced the age at which the tumor appeared.

Although the thymus in most of the estrogen-treated animals that developed lymphomas was involved in the neoplastic process this manifestation of the disease does not occur uniquely among animals so treated. Although none of the control mice in the present series showed thymic involvement spontaneous thymic tumors have been noted in mice in the Ak and Rf strains (15, 18) and in the high leukemia F strain (35). Thymic tumors developed in mice of the F strain following methylcholanthrene treatment (36, 37). Observations in this laboratory indicated that the incidence of thymic involvement in the lymphomatous process was greater in young F mice than in those that become lymphomatous at later ages. Although none of the control animals in the present series had thymic tumors the incidence of spontaneous tumors was very low among mice of these strains. The tendency for the higher incidence of mediastinal tumors among the younger mice of the lymphoma-susceptible strains was directly comparable to the similar tendency among the estrogen-treated mice. Since most of the lymphomas in control mice arose in old animals it may be supposed that normal regression of the thymus may eliminate the possibility of its involvement in the lymphomatous process, although thymic involvement was present in the lymphoid tumors occurring in old estrogen-treated mice.

In general, the application of various carcinogenic hydrocarbons increased the incidence of leukemia and also induced its development at an earlier age among genetically susceptible mice. There was not a specific morphological type of lymphoma produced by these carcinogenic agents. A wide range of types of leukemia developed in mice of one stock treated with carcinogen although the untreated controls showed relatively few spontaneous leukemias (18). Myelogenous, monocytic, and lymphosarcomatous leukoses have developed in animals subjected to x-ray and carcinogens (46). On the other hand no myelogenous or monocytic leukemias occurred following estrogen treatment. The tumors all affected the lymphoid tissue although the variations in the manifestations of the neoplasia ranged from nonmetastatic, locally invasive tumors to generalized lymphatic leukemia. Although estrogenic treatment induced the displacement of bone marrow by osseous tissue and extramedullary myelopoiesis no myelogenous leukemias occurred.

The reticular cell hyperplasia found in the spleens of many of the animals that did not have frank lymphomas indicated that this early effect of estrogen treatment might well be a precursor of lymphoid tumors. In some mice the hyperplasia was so extensive that it was difficult to distinguish it from a neoplastic process. Because all the tissues of some of these animals were not available the extent of the reticular hyperplasia could not be determined. A number of these mice may have had early malignant growths.

They were not included as lymphomatous in the present series, however, unless evidence of invasiveness was revealed. The material available was not adequate to prove that the possible "precancerous" reticular hyperplasias were more constant in those strains showing the highest incidence of lymphomas.

SUMMARY AND CONCLUSIONS

1. Eleven of 822 mice (1.34 per cent) from 7 different strains had lymphoid tumors at death. The incidence of tumors among the mice of the different strains ranged from 0 to 5 per cent.

2. The incidence of lymphoid tumors was not higher in females than in male mice. Castrated or virgin females had no more lymphomas than the intact or breeding animals. The lymphomas in untreated animals usually appeared in very old animals and did not involve the thymus.

3. Of 1,799 mice 215 (11.9 per cent) from these same strains acquired lymphomas when treated with one or more of several steroid or nonsteroid estrogens.

4. Mice of the C3H, CBA, and PM strains showed an incidence of lymphoma of approximately 15 per cent when treated with estrogens. The incidence in mice of the C12I and JK strains was approximately 5 per cent; for the A and C57 strains approximately 2 per cent.

5. Estrogen-treated female mice of the CBA and C3H strains showed almost twice as high an incidence of lymphoma as males of the same strains. The sex difference, although not so striking, was reversed in the PM and C12I strains.

6. The incidence of lymphoid tumors was highest among the groups of mice given the largest doses of estrogens; for example, only 3.4 per cent of the 267 mice of the C3H strain given small doses had lymphomatoses whereas 17.9 per cent of the 308 mice treated continuously with large doses had lymphoid tumors.

7. Mice subjected to intense estrogenic treatment for 2 to 3 months later acquired tumors more frequently (25.6 per cent of the 172 mice of the C3H strain) than did the mice treated continuously. The tumors frequently appeared after other evidence of exposure to estrogens had disappeared.

8. One estrogenic chemical was as effective as another in inducing lymphomas in mice if given in comparable doses. Large doses of the less active or more quickly eliminated estrogens such as triphenylethylene, estrone, or stilbestrol, were not so effective as smaller doses of estradiol benzoate or estradiol dipropionate or pellets of estrone, stilbestrol, or estradiol dipropionate.

9. Approximately 80 per cent of the lymphomas involved the thymus, spleen, liver, lymph nodes, or other tissues. In approximately 20 per cent the thymus and adjacent tissues alone were involved. The tumors grew when transplanted into mice of the same strain.

10. The simultaneous administration of testosterone propionate with estrogen reduced the expected incidence of lymphoid tumors (2.34 per cent of the 378 mice treated from the C3H strain) approximately to that of the untreated controls.

11. The incidence of lymphoid tumors among mice given testosterone propionate was approximately the same as in the controls.

12. The tendency for estrogens to be more effective in increasing the incidence of lymphoid tumors among some strains than among others could not be associated with any concomitant peculiarities of these strains in their response to estrogen, such as the tendency to acquire mammary, hypophyseal, or testicular tumors or the responsiveness of the skeletal tissues.

13. In increasing the incidence of lymphoid tumors in mice of some strains estrogenic hormones were as effective, if not more effective than, any other experimental procedure. The lymphoma-inducing action of estrogen was inhibited by testosterone propionate.

REFERENCES

1. Barnes, W. A., and Furth, J. A Transmissible Leukemia in Mice with Atypical Cells. Am. J.. Cancer, **30**:75-94. 1937.
2. Bischoff, F., Long, M. L., Rupp, J. J., and Clarke, G. J. Endocrine Factors Influencing Tumor Development; Effect of Gonadotropins and of Theelin upon Marsh-Buffalo Adenocarcinoma and Lymphosarcoma. Endocrinology, **28**: 769-779. 1941.
3. Bischoff, F., Long, M. L., Rupp, J. J., and Clarke, G. J. Carcinogenic Effect of Estradiol and of Theelin in Marsh-Buffalo Mice. Cancer Research, **2**: 52-55. 1942.
4. Bischoff, F., Long, M. L., Rupp, J. J., and Clarke, G. J. Influence of Toxic Amounts of Estrin upon Intact and Castrated Male Marsh-Buffalo Mice. Cancer Research, **2**:198-199. 1942.
5. Bittner, J. J. Mammary Tumors in Mice in Relation to Nursing. Am. J. Cancer, **30**:530-538. 1937.
6. Bonser, G. M., and Robson, J. M. The Effects of Prolonged Œstrogen Administration upon Male Mice of Various Strains: Development of Testicular Tumours in the Strong A Strain. J. Path. & Bact., **51**:9-22. 1940.
7. Brues, A. M., and Marble, B. B. Lymphoblastoma in Mice Following Administration of Carcinogenic Tar. Am. J. Cancer, **37**:45-53. 1939.
8. Büngeler, W. Die experimentelle Erzeugung von Leukämie und Lymphosarkom durch chronische Indolvergiftung der Maus. Frankfurt. Ztschr. f. Path., **44**:202-271. 1932.
9. Burrows, H., and Cook, J. W. Spindle-Celled Tumours and Leukaemia in Mice after Injection with a Water Soluble Compound of 1:2:5:6-Dibenzanthracene. Am. J. Cancer, **27**:267-278. 1936.
10. Cramer, W., and Horning, E. S. Hormonal Relationship Between the Ovary and the Adrenal Gland and Its Significance in the Ætiology of Mammary Cancer. Lancet, **1**:192-197. 1939.

11. Cramer, W., and Horning, E. S. Experimental Production by Œstrin of Pituitary Tumours with Hypopituitarism and of Mammary Cancer. Lancet, **1**:247-249. 1936.
12. Dougherty, T. F., Williams, W. L., and Gardner, W. U. Changes in Myeloid and Lymphoid Tissues in Estrogen Treated Dogs. Anat. Rec., **85**:19, Suppl. 1943.
13. Dougherty, T. F., and White, A. Effect of Pituitary Adrenotropic Hormone on Lymphoid Tissue. Proc. Soc. Exper. Biol. & Med., **53**:132-133. 1943.
14. Dougherty, T. F., and White, A. Influence of Adrenal Cortical Secretion on Blood Elements. Science, **98**:367-369. 1943.
15. Englebreth-Holm, J. Leukemia in Animals. London: Oliver & Boyd. 1942.
16. Engelbreth-Holm, J., and Lefèvre, H. Acceleration of the Development of Leukemias and Mammary Carcinomas in Mice by 9,10-Dimethyl-1,2,-Benzanthracene. Cancer Research, **1**:102-108. 1941.
17. Franseen, C. C., Aub, J. C., and Simpson, C. L. Experimental Tumors in Lymph Nodes and in Endocrine and Salivary Glands. Cancer Research, **1**:489-493. 1941.
18. Furth, J., and Barnes, W. A. Differences between Malignant Blood Cells From Induced and Spontaneous Leukemias of Mice. Cancer Research, **1**:17-22. 1941.
19. Furth, J., and Furth, O. B. Neoplastic Diseases Produced in Mice by General Irradiation with X-Rays. I. Incidence and Types of Neoplasms. Am. J. Cancer, **28**:54-65. 1936.
20. Furth, J., and Furth, O. B. Monocytic Leukemia and Other Neoplastic Diseases Occurring in Mice Following Intrasplenic Injection of 1:2-Benzpyrene. Am. J. Cancer, **34**:169-183. 1938.
21. Gardner, W. U. Hypertrophy of the Interstitial Cells in the Testes of Mice Receiving Estrogenic Hormones. Anat. Rec., **68**:339-347.
22. Gardner, W. U. Influence of Estrogenic Hormones on Abnormal Growths. Occas. Publ. Am. Assn. Adv. Sci. No. 4:67-75, Lancaster, Pa.: Science Press. 1937.
23. Gardner, W. U. Estrogens in Carcinogenesis. Arch. Path., **27**:138-170.
24. Gardner, W. U. Inhibition of Mammary Growth by Large Amounts of Estrogen. Endocrinology, **28**:53-61. 1941.
25. Gardner, W. U. Lymphoid Tumors in Estrogen-Treated Mice. (Abstract.) Cancer Research, **2**:725. 1942.
26. Gardner, W. U. Testicular Tumors in Mice of Several Strains Receiving Triphenylethylene. Cancer Research, **3**: 92-99. 1943.
27. Gardner, W. U., Kirschbaum, A., and Strong, L. C. Lymphoid Tumors in Mice Receiving Estrogens. Arch. Path., **29**:1-7. 1940.
28. Gardner, W. U., and Pfeiffer, C. A. Skeletal Changes in Mice Receiving Estrogens. Proc. Soc. Exper. Biol. & Med., **37**:678-679. 1938.
29. Gardner, W. U., and Pfeiffer, C. A. Inhibition of Estrogenic Effects on the Skeleton by Testosterone Injections. Proc. Soc. Exper. Biol. & Med., **38**:599-602. 1938.
30. Gardner, W. U., and Strong, L. C. Strain-Limited Development of Tumors of the Pituitary Gland in Mice Receiving Estrogen. Yale J. Biol. & Med., **12**:544-548. 1940.
31. Hartwell, J. L., and Stewart, H. L. Action of 5,9,10-Trimethyl-1,2-Benzanthracene on the Skin of the Mouse. J. Nat. Cancer Inst., **3**:277-285. 1942.
32. Hoch-Ligeti, C. Studies on the Changes in the Lymphoid Tissue of Mice Treated with Carcinogenic and Non-carcinogenic Hydrocarbons. Cancer Research, **1**:484-488. 1941.
33. Hooker, C. W., and Pfeiffer, C. A. The Morphology and Development of Testicular Tumors in Mice of the A Strain Receiving Estrogens. Cancer Research, **2**:759-769. 1942.
34. Ingle, D. J. Effect of Two Steroid Compounds on Weight of Thymus of Adrenalectomized Rats. Proc. Soc. Exper. Biol. & Med., **44**:174-175. 1940.
35. Kirschbaum, A., and Strong, L. C. Leukemia in the F Strain of Mice: Observations on Cytology, General Morphology, and Transmission. Am. J. Cancer, **37**: 400-413. 1939.
36. Kirschbaum, A., Strong, L. C., and Gardner, W. U. Influence of Methylcholanthrene and Age Incidence of Leukemia in Several Strains of Mice. Proc. Soc. Exper. Biol. & Med., **45**:287-289. 1940.
37. Kirschbaum, A., and Strong, L. C. Influence of Carcinogens on the Age Incidence of Leukemia in the High Leukemia F Strain of Mice. Cancer Research, **2**: 841-845. 1942.
38. Krebs, C., Rask-Nielsen, H. C., and Wagner, A. The Origin of Lymphosarcomatosis and Its Relation to Other Forms of Leucosis in White Mice. Acta. radiol., suppl 10: 1-53. 1930.
39. Lacassagne, A. Sarcomes Lymphoïdes apparus chez des Souris longuement traitées par des hormones oestrogènes. Comp. rend. Soc. de biol., **126**:193. 1937.
40. Lacassagne, A. Statistique des différents cancers constatés dans des lignées sélectionnenées de souris, aprés action prolongée d'hormones oestrogènes. Bull. Assoc. franç. p. l'étude du cancer, **27**:96-115. 1938.
41. Law, L. W. The Induction of Leukemia in Mice Following Percutaneous Application of 9,10-Dimethyl-1,2-Benzanthracene. Cancer Research, **1**:564-571. 1941.
42. Lawrence, J. H., and Gardner, W. U. A Transmissible Leukemia in the "A" Strain of Mice. Am. J. Cancer, **33**: 112-119. 1938.
43. Lignac, G. O. E. Die Benzolleukämie bei Menschen und Weissen Mäusen. Klin Wchnschr., **1**:109-110. 1933.
44. Loeb, L. The Significance of Hormones in the Origin of Cancer. J. Nat. Cancer Inst., **1**:169-195. 1940.
45. MacDowell, E. C. Genetic Aspects of Mouse Leukemia. Am. J. Cancer, **26**:85-101. 1936.
46. McEndy, D. P., Boon, M. C., and Furth, J. Induction of Leukemia in Mice by Methylcholanthrene and X-rays. J. Nat. Cancer Inst., **3**:227-247. 1942.
47. Mercier, L. Dystrophies oculaires et tumeurs chez des Souris après injections intrapéritonéale de goudron. Compt. rend. Soc. de biol., **102**:503-505. 1929.
48. Mercier, L. Lymphadénome expérimental de la Souris obtenu par injection intrapéritonéale de goudron de houille. Compt. rend. Soc. de biol., **103**:125-126. 1930.
49. Mider, G. B., and Morton, J. J. The Effect of Methylcholanthrene on the Latent Period of Lymphomatosis in Dilute Brown Mice. Am. J. Cancer, **37**:355-363. 1939.
50. Moon, H. D. Effect of Adrenocorticotropic Hormone in 4-Day-Old Rats. Proc. Soc. Exper. Biol. & Med., **43**: 42-44. 1940.
51. Morton, J. J., and Mider, G. B. Some Effects of Carcinogenic Agents on Mice Subject to Spontaneous Leukoses. Cancer Research, **1**:95-98. 1941.
52. Perry, I. H., and Ginzton, L. L. The Development of Tumors in Female Mice Treated with 1:2:5:6-Dibenzanthracene. Am. J. Cancer, **29**:680-704. 1937.
53. Richter, M. N., and MacDowell, E. C. Experiments with Mammalian Leukemia. Physiol. Rev., **15**:509-524. 1935.
54. Schacher, J., Browne, J. S. L., and Selye, H. Effect of Various Sterols on Thymus in the Adrenalectomized Rat. Proc. Soc. Exper. Biol. & Med., **36**:488-491. 1937.

55. Selye, H. Thymus and Adrenals in the Response of the Organism to Injuries and Intoxications. Brit. J. Exper. Path., **17**:234-248. 1936.

56. Selye, H., Collip, J. B., and Thomson, D. L. Effect of Oestrin on Ovaries and Adrenals. Proc. Soc. Exper. Biol. & Med., **32**:1377-1381. 1935.

57. Selye, H., Harlowe, C. M., and Collip, J. B. Über die Auslösung der Alarmreaktion mit Follikelhormon. Endokrinologie, **18**:81-87. 1936.

58. Shimkin, M. B., Grady, H. G., and Andervont, H. B. Induction of Testicular Tumors and Other Effects of Stilbestrol-Cholesterol Pellets in Strain C Mice. J. Nat. Cancer Inst., **2**:65-80. 1941.

59. Strong, L. C. The Origin of Some Inbred Mice. Cancer Research, **2**:531-539. 1942.

60. Tyslowitz, R., and Dingemanse, E. Effect of Large Doses of Estrogen on the Blood Picture of Dogs. Endocrinology, **29**:817-827. 1941.

61. Tepperman, T., Engel, F. L., and Long, C. N. H. A Review of Adrenal Cortical Hypertrophy. Endocrinology, **32**:373-402. 1943.

62. Weichert, C. K., and Hale, H. B. Inhibition of Estrogenic Effects on the Reproductive System of the Male Rat by Testosterone Injections. Endocrinology, **33**:16-22. 1943.

63. Wilson, J. G. Reproductive Capacity of Adult Female Rats Treated Prepuberally with Estrogenic Hormone. Anat. Rec., **86**:341-364. 1943.

Attempts to Induce Stomach Tumors

II. The Action of Carcinogenic Hydrocarbons on Stock Mice*

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Thirty years ago Fibiger (4) described experimentally induced papilloma of the forestomachs of rats and mice, and also one transplantable squamous carcinoma in the forestomach of a mouse; these occurred in animals maintained on a diet of white bread and water, and infested with a round worm, *Gongylonema neoplasticum,* of which the intermediate host was the cockroach. Fibiger's experiments involved many uncontrolled factors, notably deficiency of vitamin A in the diet, the significance of which could not be appreciated in 1915. Since then many workers have tried by various means to induce malignant tumors in the stomachs of rodents, but for the most part without success. Recent reviews of the literature have been published by Klein and Palmer (6), Sugiura (16), and Kirby (5).

The advent of pure hydrocarbons as carcinogens led to renewed attempts to induce tumors of the alimentary canal in animals. In 1936 Oberling and his associates (10), and Waterman (19) independently, obtained malignant tumors of the forestomach in mice receiving 3,4-benzpyrene orally. Few positive results were reported, however, and no stomach tumors were obtained in mice fed with 1,2,5,6-dibenzanthracene for 6 months (8,18), while only squamous papillomas were obtained in mice fed 20-methylcholanthrene for the same length of time (8). More recent successes in the induction of gastric tumors following the injection of carcinogens into the stomach wall by Stewart and Lorenz (14) offer additional evidence of the susceptibility of the gastric mucosa of mice to the action of recognized carcinogens but, as they involve a different procedure, will not be considered here. The experiments now reported form part of an investigation of alimentary cancer begun in 1939 in this laboratory. The induction of benign gastropapillomatosis in rats maintained on an adequate diet to which were added fats previously heated to the maximum temperatures used in domestic cooking, has already been reported (2). It was shown that this result was due to avitaminosis A induced by interference with vitamin A metabolism by some factor present in heated fats. The lesions were benign and could be prevented or cured by feeding the rats with extra rations of raw carrots, in excess of the amount required to maintain control animals receiving similar quantities of unheated fats in normal health. Mice fed in the same way rarely developed gastric lesions, but two cases of papillomatosis and one of hyperkeratosis were recorded amongst 45 mice surviving more than 4 months.

Attempts to induce tumors of the *pars glandularis* have met with so little success that it should be mentioned that Stewart and Lorenz (14) reported adenocarcinoma in C3H male mice following injection into the wall of the pyloric chamber of a suspension of methylcholanthrene in horse serum. Recently, Strong, Collins, and Durand (15) have described adenocarcinoma of the stomach in a few NHO mice after subcutaneous injection of methylcholanthrene. Spontaneous gastric tumors, whether of the *pars squamosa* or *pars glandularis,* are known to be extremely rare in stock mice, though adenomas have been reported in Strain 1 mice (13). Stock mice are therefore suitable animals on which to test potential carcinogens added to the diet. Since 20-methylcholanthrene is shown to be a stronger carcinogen than is 3,4-benzpyrene for the skin and connective tissues of mice (1, 9, 11, 12) it was thought that other factors might be responsible for the difference between the results of Oberling and his group (10) and those of van Prohaska, Brunschwig, and Wilson (18), and our series was designed to test both carcinogens under identical conditions. To ascertain whether the addition of vitamin A to the diet would influence the effects of such feeding, the hydrocarbons were administered to some mice in olive oil and to others in cod liver oil.

EXPERIMENTAL

Four groups of 12 stock mice, of mixed colors, were kept on wood shavings in metal boxes; they received a diet of rat-cake (17), and water *ad libitum.* The hydrocarbons, dissolved in either olive oil or cod liver oil, were administered by allowing single drops of

* Because of the difficulties of international communication the authors have not read proof of this article.

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the oily solutions to soak into small pieces of rat-cake, which were given to the mice early in the morning when they were hungry and would consume their appropriate dose promptly. The distribution of the hydrocarbons and solvents between the groups is given in Table I.

TABLE I

Group	Hydrocarbon	Solvent	Strength
I	3,4-benzpyrene	Olive oil	0.4%
II	"	Cod liver oil	"
III	20-methylcholanthrene	Olive oil	"
IV	"	Cod liver oil	"

A control group of 6 mice from the same stock was similarly housed and fed but with the addition of plain cod liver oil in place of hydrocarbon solution.

RESULTS

In the control group 4 mice survived more than 100 days. Slight diffuse hyperkeratosis and slight hyperplasia without papilloma formation were seen in 1 dying on the 113th day; slight diffuse hyperkeratosis only was found in 1 dying on the 426th day; the other 2 survivors died on the 487th and 545th days respectively, but showed no abnormality of the gastric mucosa. No lesions were seen in any other parts of the alimentary tract in any of these mice. Slight degrees of diffuse hyperkeratosis and hyperplasia, amounting to about double the thickness seen in most sections of mouse stomach, have often been encountered in examining many mouse stomachs in this Department, not only in this experiment, and little importance should be attached to such findings as an indication of carcinogenic action. Such minor degrees of hyperplasia resemble, in general, the more advanced hyperplasia and hyperkeratosis that seemed to be the prelude to generalized gastropapillomatosis seen in earlier experiments in rats and mice, to which reference has already been made. Their general appearance is quite different from the potentially malignant and often strictly localized papillomas induced by carcinogenic hydrocarbons and described below.

The results in the groups receiving hydrocarbons are summarized in Table II, in which lesions of the forestomach only are tabulated, as no significant lesions were observed in the *pars glandularis*. The degree of hyperkeratosis, hyperplasia, and papillomatous growth are indicated very approximately by + signs; one + indicating not more than treble the normal thickness of the keratinized or cellular layers, respectively, under the headings "Hyperkeratosis" and "Hyperplasia"; ++ a more definite degree of thickening, while +++ indicates a still more decided thickening. The relative amounts of papillomatous growth are difficult to estimate and record, but a single + indicates a distinct papilloma with connective tissue and vascular core without evidence of branching. Further + signs indicate branching and/or multiple papillomas, which, however, vary considerably in size. On the basis of our histological experience, more importance is attached to the morphology of papillomas than to their volume. Characteristic appearances are illustrated in the accompanying photomicrographs.

In group I, the first papilloma was observed in an animal dying on the 346th day of the experiment; 10 animals survived longer than this; 7 altogether had papillomas of the forestomach and in 2 of these the histological appearances suggested early malignancy. In group II, the first papilloma was observed in an animal dying on the 158th day of the experiment; 6 animals survived longer than this; 4 altogether had papillomas of the forestomach and one of these showed signs of malignancy. In group III, the only papilloma was observed in an animal dying from lung abscesses on the 256th day of the experiment; 6 others survived for longer periods, but showed no significant gastric lesions. In group IV, the first papilloma was observed in an animal dying on the 316th day; 5 survived for longer periods; altogether 5 showed papillomas, 3 with signs of malignancy.

In our experiments, only minor pathological changes have been seen in the glandular part of the stomachs. These are, for the most part, punctate erosions involving almost the whole thickness of the glandular epithelium but usually leaving the fundi of the glands intact. Complete regeneration of such epithelium seems to be possible. Small numbers of cystic glands have been seen in several animals, but these have been unassociated with obvious pathological processes and appear to be congenital defects similar, in a lesser degree, to the adenomatous congenital tumors seen in I strain mice (13).

DISCUSSION

Although hyperplastic lesions and benign tumors of the stomachs of rats and mice have been ascribed to a great variety of agencies, assertions that squamous carcinoma have been induced have been relatively rare. Until quite recently there have been no reports that adenocarcinoma of the stomach had been elicited. At the present time the only established method of inducing malignant tumors of either part of the mouse stomach is to expose the mucous membrane to the action of a recognized carcinogenic hydrocarbon. Waterman (19, 20) wrote that he had induced metastasizing squamous cell carcinoma of the mouse by giving 3,4-benzpyrene orally as a 4 per cent solution in olive oil. Oberling and his associates (10) described squamous epithelioma induced in 2 out of 3 mice

that survived weekly feeding with 3,4-benzpyrene in lard for 6 months. Van Prohaska, Brunschwig, and Wilson (18) observed squamous papilloma in 2 mice fed with 1 per cent 20-methylcholanthrene for 186 days. Lorenz and Stewart (7) obtained squamous carcinoma in 10 mice of strain A fed with olive or mineral oil solutions of methylcholanthrene for 7 months, but failed to induce similar lesions in strains C3H, C57 black, and C57 brown with such solutions; 1,2,5,6-dibenzanthracene was ineffective in all four strains. Recently, Collins, Gardner, and Strong (3) have described transplantable squamous carcinoma of the forestomach in C57 black female mice fed with 0.3 mgm. 3,4-benzpyrene twice weekly for an average of 357 days. C3H mice of both sexes developed similar lesions after 300 days, and spayed C3H mice after

TABLE II

Group	Duration of hydrocarbon feeding	Lesions of forestomach			Number showing papillomas	
		Hyperkeratosis	Hyperplasia	Papillomatosis	Before 300 days	After 300 days
I	63 days	+	0	0	0/1	
	346 "	++	+	+		
	350 "	+++	+	+		
	388 "	+++	++	++		
	412 "	+	0	0		
	432 "	++	+	+		
	458 "	0	0	0		7/11
	472 "	0	0	0		
	484 "	+	0	0		
	486 "	++	++	++		
	498 "	++	++	++		
	508 "	++	+++	++		
II	46 "	+	0	0		
	46 "	+	0	0		
	102 "	+	+	0		
	103 "	+	+	0	1/8	
	104 "	+	0	0		
	158 "	+++	++	++		
	248 "	+	0	0		
	298 "	0	0	0		
	308 "	0	0	0		
	322 "	+++	++	++		
	434 "	+++	++	++		3/4
	571 "	+++	++	++		
III	79 "	+	0	0		
	86 "	++	+	0		
	139 "	0	0	0		
	252 "	+	0	0		
	239 "	+++	0	slight	1/9	
	256 "	++	+	+		
	264 "	0	0	0		
	280 "	0	0	0		
	286 "	+	0	0		
	315 "	+	0	0		
	329 "	+	0	0		0/3
	509 "	+	+	0		
IV	90 "	0	0	0		
	155 "	0	0	0		
	202 "	+	0	0	0/4	
	245 "	+	0	0		
	248 "	—	—	—		
	316 "	+++	++	++		
	320 "	++	+	tendency		
	334 "	0	+	0		4/6
	386 "	+++	++	+++		
	393 "	+++	++	++		
	393 "	++	+	+		

an average of 267 days. NH females developed papillomatosis, but spayed females of this strain developed carcinomas at an average of 350 days, as a result of similar treatment. The mechanism of this cocarcinogenic effect of spaying is not clear, but, as far as mice of the C3H and NH strains are concerned, spaying seems to increase the tendency for gastric papillomas to become malignant. If this effect of spaying had a general application for mice, one would have expected benzpyrene to induce at least as many stomach carcinomas when fed to spayed mice of strain A as did methylcholanthrene in intact mice of this strain (7). Actually, Collins, Gardner, and Strong obtained no carcinomas at all in spayed A mice fed benzpyrene up to 350 days. Thus the relative carcinogenic potencies of 3,4-benzpyrene and of 20-methylcholanthrene seem to be completely reversed in strain A mice on the one hand, and in C3H and C57 black mice on the other.

That the use of pure strains of mice in such experiments may lead to very different results is not surprising when one considers the variable behavior shown by mixed populations of stock mice subjected to the action of a carcinogen. In investigating a problem that is primarily concerned with human pathology, it is well to bear in mind that there are no inbred lines of human beings, and that experiments carried out in purebred animals may have a very limited bearing on the etiology of malignant neoplastic disease in corresponding sites in human beings or even in laboratory animals of mixed stock. The initial determination of the carcinogenicity of any substance is best carried out in animals of a mixed stock, though comparisons between certain carcinogens can be expressed quantitatively if pure lines of animals are used for their assay, with the proviso that generalizations from such results are not warranted. Even the striking experiments of Stewart and Lorenz (14) indicate only that the glandular part of the stomach of C3H mice, and possibly of other pure strains, is susceptible to the action of known carcinogens but give no information as to the capacity of the average mouse stomach to respond to a similar stimulus. The small series of experiments here described shows that the stock mice used in this laboratory for investigating the possible dietary carcinogenic factors are capable of developing papillomas, some of which are histologically malignant, in response to the action of known carcinogenic hydrocarbons. Such results are thought to be significant in view of the absence of any such lesion amongst many hundreds of mice used in other experiments in this laboratory. The present position seems to be that benign tumors of the forestomach of rodents fall into two categories, one containing tumors that tend to remain benign, though frequently extensive; the other, tumors that are essentially malignant in their evolution, though frequently very localized. We find it convenient to describe the former by the term "gastropapillomatosis," and to reserve the expression "papilloma" and "carcinoma" for the different stages in the evolution of the latter type. A number of dietary factors seem to be capable of inducing gastropapillomatosis, but at the present time malignant tumors can be induced only by the action of carcinogenic hydrocarbons.

SUMMARY

1. Stock mice receiving an adequate diet with the addition of either 3,4-benzpyrene or 20-methylcholanthrene in solution in olive oil or cod liver oil developed papillomas of the forestomach, with tendency to malignant evolution. Even a small number of positive results in the experimental induction of tumors of the forestomach of mice must be considered significant in view of the extreme rarity of such tumors in this species.

2. Unlike the diffuse benign gastropapillomatosis associated with dietary deficiencies, papillomas induced with carcinogenic hydrocarbons (a) tend to be localized and invasive, and (b) are not prevented or influenced by the addition of large amounts of vitamin A to the diet.

3. The relative values of stock mice and inbred lines for investigating the competence of tissues to react to carcinogens are discussed.

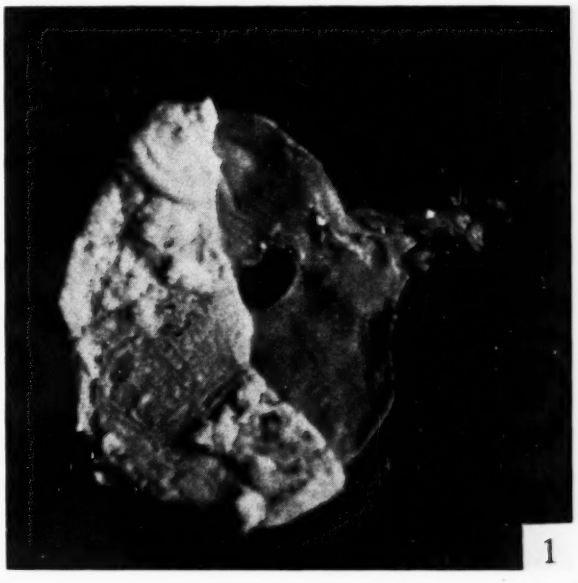
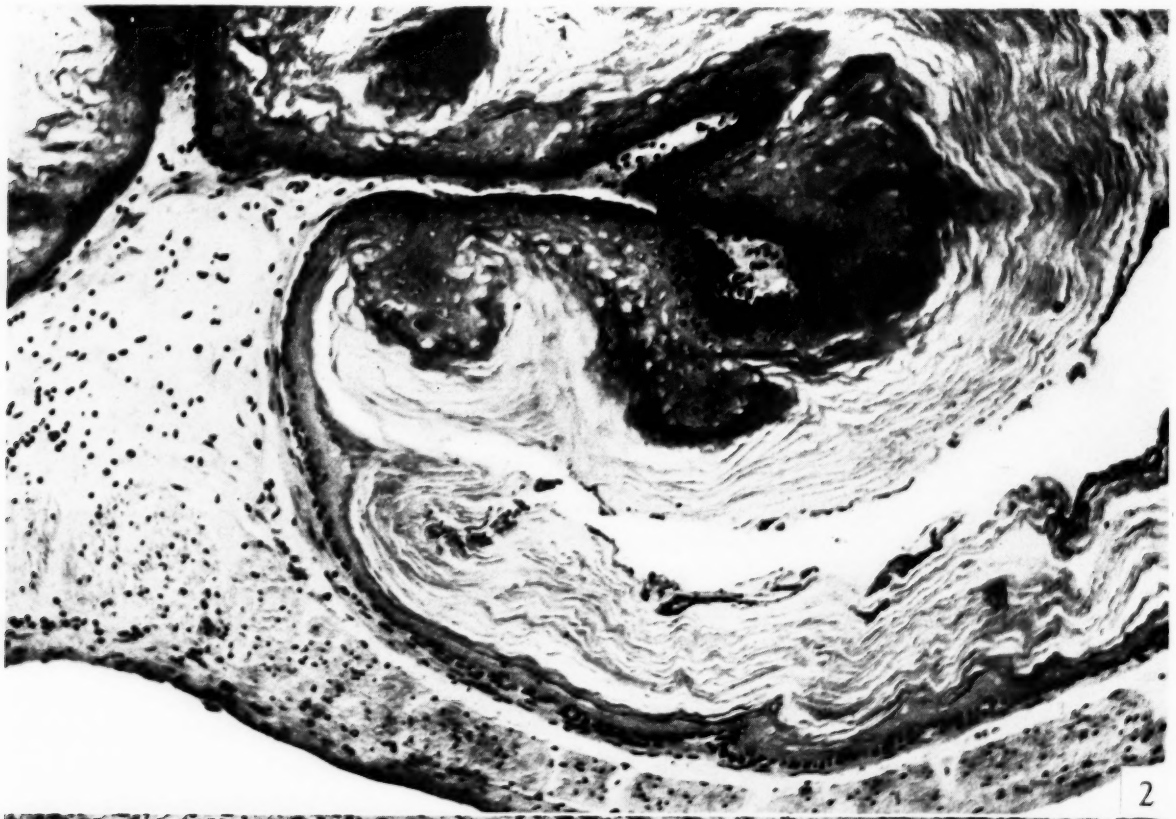


FIG. 1.—Naked eye appearance of stomach of mouse A3, fed for 13 months. Gross lesions limited to forestomach, which presents many papillomas.



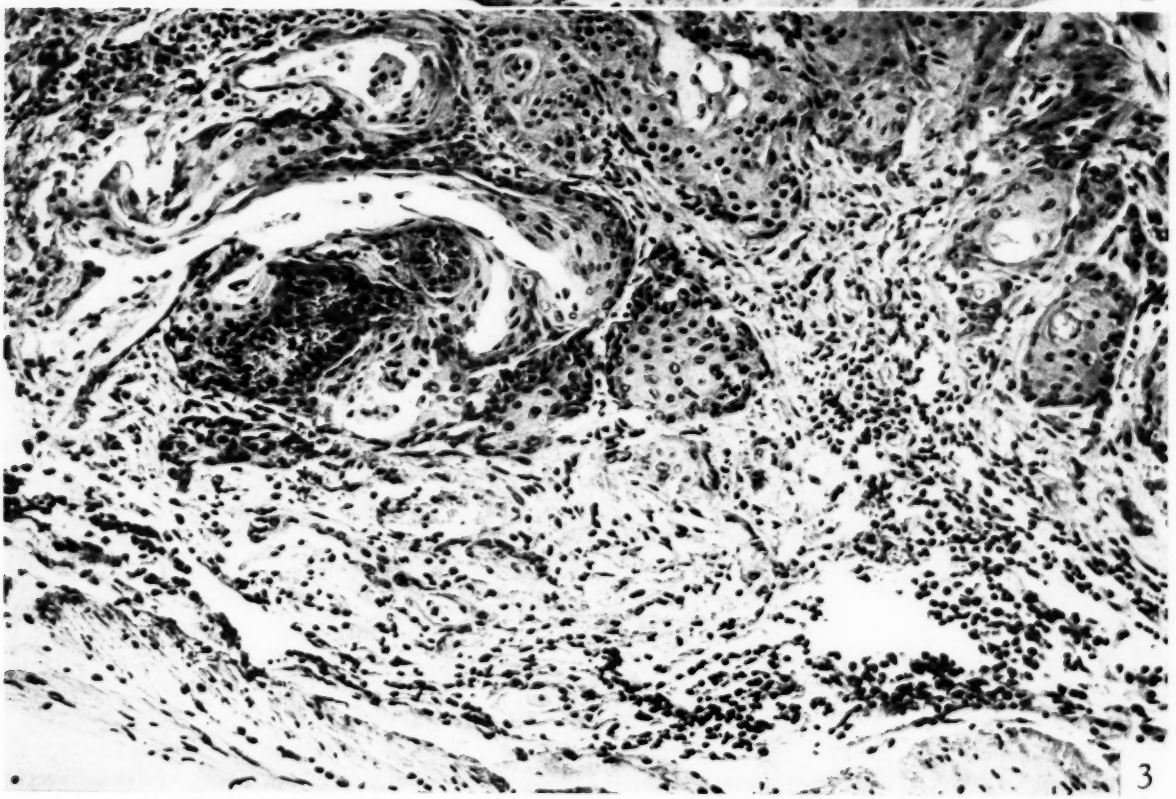


FIG. 2.—Part of branching papilloma of forestomach of mouse A3, showing well developed stroma of connective tissue in papilloma and hyperkeratosis of neighboring gastric epithelium.

FIG. 3.—Part of base of papilloma of forestomach of mouse H6, fed for 13 months. The tumor infiltrates through the *muscularis mucosae* but does not invade the muscle of the stomach wall. Histologically, this is a squamous carcinoma.

REFERENCES

1. Barry, G., Cook, J. W., Haselwood, G. K., Hewitt, C. L., Hieger, I., and Kennaway, E. L. Production of Cancer, by Pure Hydrocarbons. Part III. Proc. Roy. Soc., London, s.B., **117**:318-351. 1935.
2. Beck, S., and Peacock, P. R. Gastro-Papillomatosis Due to Vitamin A Deficiency Induced by Heated Fats. Brit. M. J., **2**:81-83. 1941.
3. Collins, V. J., Gardner, W. U., and Strong, L. C. Experimental Gastric Tumors in Mice. Cancer Research, **3**:29-35. 1943.
4. Fibiger, J. Untersuchungen über eine Nematode (Spiroptera sp. n.) und deren Fähigkeit papillomatöse und Carcinomatöse Geschwulstbildungen im Magen der Ratte hervorzurufen. Ztschr. f. Krebsforsch.,**13**:217-280. 1913.
5. Kirby, A. H. M. Attempts to Induce Stomach Tumors. I. The Effect of Cholesterol heated to 300° C. Cancer Research, **3**:519-525. 1943.
6. Klein, A. J., and Palmer, W. L. Experimental Gastric Carcinoma: A Critical Review with Comments on the Criteria of Induced Malignancy. J. Nat. Cancer Inst., **1**:559-584. 1941.
7. Lorenz, E., and Stewart, H. L. Squamous Cell Carcinoma and Other Lesions of the Forestomach, in Mice, Following Oral Administration of 20-Methylcholanthrene and 1, 2, 5, 6-Dibenzanthracene. J. Nat. Cancer Inst., **1**:273-276. 1940.
8. Magnus, H. A. The Experimental Production of Malignant Papillomata of the Lung in Mice with 1,2,5,6-Dibenzanthracene. J. Path. & Bact., **49**:21-31. 1939.
9. Nicod, J. L., and Regamey, J. Les cancers provoqués par le méthylcholanthrène chez la souris. Bull. Assoc. franç. p. l'étude du cancer, **27**:706-722. 1938.
10. Oberling, C., Sannié, C., Guérin, M., and Guérin, P. Recherches sur l'action cancérigène du 1,2 benzopyrène. Bull. Assoc. franç. p. l'étude du cancer, **25**:156-180. 1936.
11. Shear, M. J. Studies in Carcinogenesis. I. The Production of Tumors in Mice with Hydrocarbons. Am. J. Cancer, **26**:322-332. 1936.
12. Shimkin, M. B., and Andervont, H. B. Comparative Carcinogenicity of Three Carcinogenic Hydrocarbons. J. Nat. Cancer Inst., **1**:57-62. 1940.
13. Stewart, H. L. Hyperplastic and Neoplastic Lesions of the Stomach in Mice. J. Nat. Cancer Inst., **1**:489-502. 1941.
14. Stewart, H. L., and Lorenz, E. Induction of Adenocarcinoma of Pyloric Stomach in Mice by Methylcholanthrene. J. Nat. Cancer Inst., **2**:193-196. 1941.
15. Strong, L. C., Collins, V. J., and Durand, E. A. A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. IV. The Probable Remote Induction of Various Types of Gastric Lesions. Cancer Research, **3**:21-28. 1943.
16. Sugiura, K. The Relation of Diet to the Development of Gastric Lesions in the Rat. Cancer Research, **2**:770-775. 1942.
17. Thomson, W. Stock Diet for Rats. J. Hyg., **36**:24-25. 1936.
18. van Prohaska, J., Brunschwig, A., and Wilson, H. Oral Administration of Methylcholanthrene to Mice. Arch. Surg., **38**:328-333. 1939.
19. Waterman, N. Experimental Production of Carcinoma in the Stomach of Mice. Acta Cancerologica, **2**:375-388. 1936.
20. Waterman, N. Production of Carcinoma of the Stomach in Mice by 1.2.-Benzopyrene. Acta brev. Neerland., **7**:18-20. 1937.

Attempts to Induce Stomach Tumors

III. The Effects of (a) A Residue of Cholesterol Heated to 300° C., and (b) △3,5-Cholestadiene*

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In recent years Roffo has described (8-11) the production of gastropapillomatosis of the forestomach, adenocarcinoma of the glandular stomach, and mesenteric and hepatic sarcoma in rats by feeding various fats or cholesterol heated to 350° C. for half an hour. Results obtained in this laboratory by feeding heated fats (1, 7) and heated cholesterol (5) to Wistar rats have already been published. No adenocarcinomas have occurred in our experiments; the feeding of heated fats led to an induced avitaminosis A associated with papillomatosis of the forestomach, but no hyperplastic lesions were seen in either part of the stomach after ingestion for 2 years of cholesterol heated to 300° C. for half an hour. The noncarcinogenicity of cholesterol heated to 200° C. or to 300° C. has been demonstrated also by Steiner, Steele, and Koch (13) who found no tumors up to 18 months when the heat products were injected subcutaneously into mice.

(a) Experiments with the Residue

The possibility arose of increasing any carcinogenic activity in heated cholesterol by the elimination of some noncarcinogenic products of heating. It was observed in early preparations of heated cholesterol that the molten mass, after having been heated for half an hour to 300° C., was no longer entirely soluble in ethanol. If the suspension was filtered hot, a white substance could be separated that had a melting point of about 192° C. and gave a carmine-red color with antimony trichloride in chloroform solution only on long standing. It was at first thought that this substance would be the β-cholesterol that Diels and Linn (2) isolated from cholesterol heated in the same way but poured into acetone instead of ethanol. This compound, however, was supposed to melt at 160° C. It was subsequently noted that Mauthner and Suida (6) had obtained an ethanol-insoluble derivative from cholesterol by heating with anhydrous copper sulphate to 200° C. This compound melted at 188° to 192° C. and was dicholesteryl ether, formed by elimination of 1 molecule of water between 2 molecules of cholesterol. Since, moreover, dicholesteryl ether was shown by Wokes (17) to give a red color with antimony trichloride in chloroform solution only on long standing, it was concluded that the compound obtained by pyrolysis of cholesterol at 300° C. was dicholesteryl ether. Thus one substance of known constitution could easily be removed from the mixture of heat products.

Diels and Linn (2) asserted that 50 per cent of the cholesterol that they heated to 310° C. for half an hour was converted to cholestenone. Heilbron and Sexton (4) also obtained this ketone by distillation of cholesterol. This compound was therefore reasonably certain to be present in the mixture of heat products. Diels and Linn used cold methanol to extract the cholestenone and this procedure was adopted here.

A quantitative experiment showed that, on average, 84 per cent of the cholesterol was recoverable unchanged at any one heating. According to Diels and Linn (3), at least 75 per cent was converted to cholestenone and "β-cholesterol," while Roffo described total destruction of cholesterol heated to 350° C. for half an hour. Actually, in our quantitative experiment, about 8 per cent of the cholesterol used was isolated as dicholesteryl ether; nothing separated from the ethanol solution on cooling, *i.e.*, no "β-cholesterol" was obtained. The weight of Δ4-cholestenone obtained corresponded to 32 per cent of the original cholesterol.

Feeding of residue.—The methanol-insoluble residue was a dark red translucent gum. It was dissolved in chloroform to make a 20 per cent solution, and this solution was dropped on to rat-cake (15), 0.1 ml. on each piece, as described in Part I (5). One piece of this rat-cake, *i.e.*, 20 mgm. of residue, was fed daily to each of a group of 24 Wistar rats. These rats also

* Because of the difficulties of international communication the author has not read proof of this article.

** The author wishes to express his gratitude for encouragement and criticism from Dr. P. R. Peacock, who also carried out the histological examinations.

received an adequate supply of untreated rat-cake and water *ad libitum*, while milk and green-stuff were fed once weekly.

Results with residue.—Four of the 24 rats died before 200 days. Of the rest, the first died at 405 days; 7 others before 500 days; 10 between 500 and 600 days, and the remainder at 653 and 687 days respectively. Of these 20 rats, one died from pneumonia; the remainder all had more or less severe lung abscesses. The hemorrhagic erosions reported in Part I were also frequently seen here and presumably are not related to the feeding of the residue. In the 20 rats surviving 400 days, all except 3 had normal forestomachs. The rat dying of pneumonia at 547 days showed a slight, unevenly distributed hyperkeratosis; one dying at 548 days showed a generalized hyperkeratosis with a slight hyperplasia tending to form minute papillae; that dying at 653 days showed an area of localized hyperkeratosis and hyperplasia, but the last rat to die, at 687 days, showed no abnormality of the forestomach. A condition of generalized slight hyperkeratosis and hyperplasia was seen in one rat fed whole heated cholesterol (5); this minor degree of change was seen in 3 of 12 controls and cannot be ascribed to the heat products of cholesterol.

(b) Experiments with Δ3,5-Cholestadiene

Beside the intermolecular dehydration of cholesterol to form dicholesteryl ether, intramolecular dehydration might occur to yield one or more cholestadienes. According to Staveley and Bergmann (12), a cholestadiene, m.p., 79° C., arises on heating cholesterol with kieselguhr or on heating cholesteryl phosphates; this substance they demonstrated to be Δ3,5-cholestadiene. Moreover, Heilbron and Sexton (4) regarded the formation of pseudocholestene on distilling cholesterol at normal atmospheric pressure as due to dehydration of the cholesterol to cholesterilene, which was then dehydrogenated to pseudocholestene. Δ3,5-Cholestadiene gives an immediate carmine-red color with antimony trichloride in chloroform and the products of heating cholesterol to 300° C. were tested in this way for the diene. Several fractions, especially first filtrates from chromatograms, gave an immediate color, but it was not found possible to isolate any cholestadiene.

The absence of any considerable quantity of Δ3,5-cholestadiene in the products of heating cholesterol to 300° C. was rather against the theory advanced by Veldstra (16) that the effective agent in heated fats or heated cholesterol was this particular diene. It was observed in this Department by Beck (1) that lard heated to 330° C. for half an hour gave a brownish red color with antimony trichloride in chloroform. It seemed possible that this color was due to a diene but masked by the colored material in heated fat. A quantity of heated lard was, therefore, saponified and the nonsaponifiable fraction tested for dienes. However, only a brownish red color could be obtained, even with the early chromatogram filtrates, which would have contained any diene present in the fraction. It seems unlikely, therefore, that any diene is formed in lard by heating to 330° C.

Strong's work (14) would seem to indicate that neither Δ3,5- nor Δ2,4-cholestadiene is a powerful carcinogen, since no tumors were obtained by painting or by injecting either of these hydrocarbons. Hence the presence of traces of either diene could hardly account for the lesions that Roffo described. However, Veldstra (16) states that Waterman obtained 4 papillomas of the stomach in an unspecified number of mice by feeding Δ3,5-cholestadiene for one year, and it seemed desirable to test this substance by mouth in rats.

Feeding Δ3,5-cholestadiene.—Δ3,5-Cholestadiene was prepared by the method of Eck and Hollingsworth (3) from cholesterol, which was either nonfluorescent (in chloroform solution) or had been rendered nonfluorescent by purification *via* the acetate. A product melting at 79° to 80° C. and having $[\alpha]_{D}^{R.T.} - 110°$ or greater is readily prepared by refluxing 50 gm. cholesterol in 200 ml. xylene with 50 gm. anhydrous copper sulphate for 4 hours, filtering, and transferring the product to petroleum ether (b.p., 60° to 80° C.) solution, passing this solution through a large column of activated alumina, to remove other substances, and recrystallizing the diene from ethanol.

The diene was dissolved in chloroform to give a 25 per cent solution and was dropped on to rat-cake, as previously described for the heated cholesterol solutions. One piece of impregnated rat-cake, *i.e.*, 25 mgm. of diene, was fed daily to each of 24 Wistar rats maintained on the same basal diet as was used in previous experiments. In case this diene was the substance responsible for the induced avitaminosis A seen in rats fed fats heated to 330° C. (1), half this group of rats received a supplement of carrot, about 2 gm. per rat, 3 times a week.

Results with Δ3,5-cholestadiene.—No tumors were seen in any rats. Apart from the usual hemorrhagic erosions of the glandular zone, the only abnormality seen in the stomach was some generalized hyperkeratosis in rat 70, dying at 416 days, and in rat 72, dying at 449 days, both of which had received the supplements of carrot. No tendency to papillomatosis was seen in the group receiving no carrot, although 4 of these died between 400 and 500 days, and 2 between 500 and 600 days, while 6 survived more than 600 days, the last being sacrificed on account of poor

condition at 689 days. In the carrot-fed group, 7 rats outlived those showing hyperkeratosis, the last dying at 691 days. Rat 72 was the first rat seen in this Department to have a stone in the bladder; this will be described below.

There is thus no evidence that Δ3,5-cholestadiene has any injurious effect on the stomachs of rats when fed at the relatively high level of 25 mgm. daily for nearly 2 years. Moreover, there was no evidence of consistent damage to organs other than the stomach, although in some rats the kidneys showed gross damage of long standing with fibrosis and the presence of eosinophil casts.

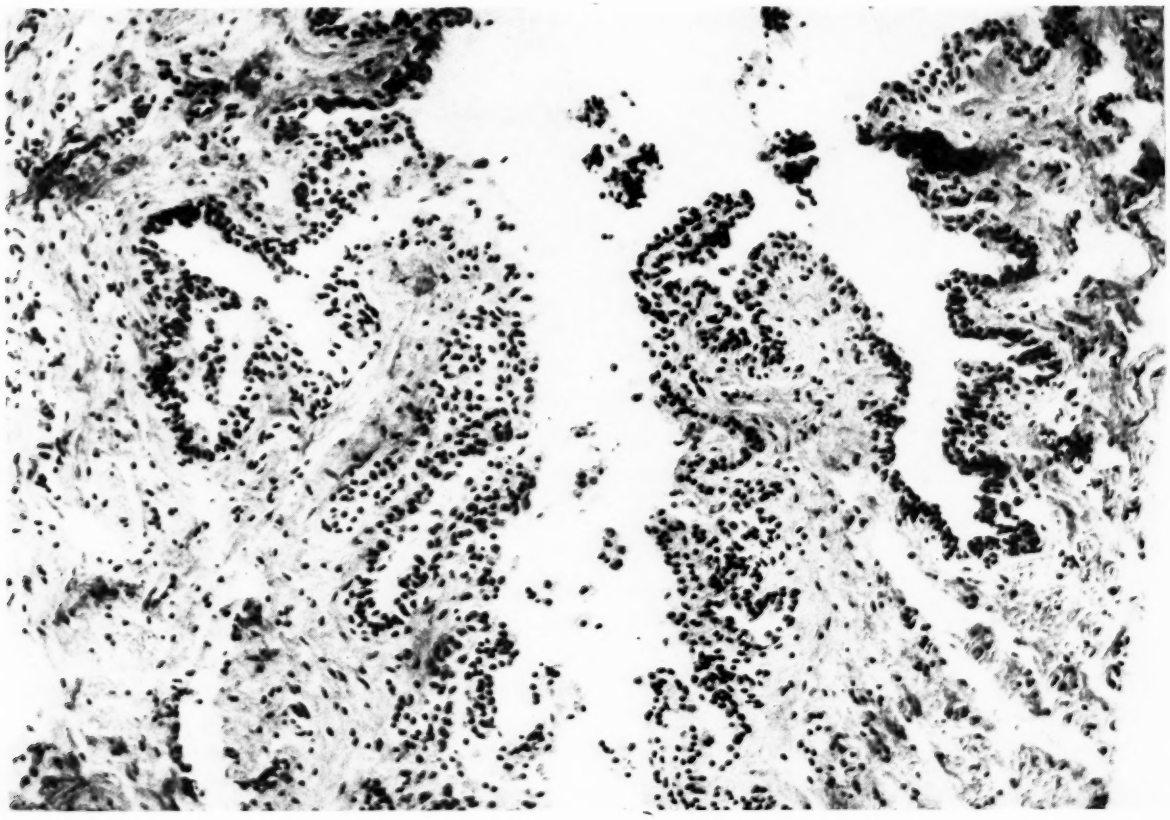

FIG. 1.—Bladder. Pseudo-papillomatous hyperplasia of mucous membrane accompanied by catarrhal shedding of surface layers of transitional epithelium in bladder of rat 72, which contained a single stone. There was no evidence of neoplasia in any part of this bladder.

Rat 72.—This rat died at approximately 18 months of age, after 440 days' feeding with Δ3,5-cholestadiene. All organs appeared normal to the naked eye except the kidneys. The bladder contained a large, smooth stone measuring 17 mm. along its greatest axis, and 13 mm. and 9 mm. respectively in the other two planes; the weight was 1.83 gm.

Analyses.—(1) Qualitative: The stone proved to be entirely inorganic; no urates, carbonates, oxalates, cystine, or cholesterol were present.

(2) Quantitative: [1]

Loss of weight on ignition	= 54.56	per cent
Proportion of calcium as CaO	= 1.69	" "
Magnesium as MgO	= 15.04	" "
Phosphorus as P_2O_5	= 29.90	" "
	101.19	" "

Nitrogen as NH_3 (microkjeldahl) = 5.49 per cent
There was also a trace of iron.

The analysis corresponds very closely to that for $Mg\ NH_4\ PO_4 \cdot 6H_2O$ in which, however, part of the magnesium has been replaced by calcium.

The bladder itself showed loss of mucosa, presumably due to irritation by the stone. Submucous fibrosis was present and numerous papillae (not neoplastic) projected into the lumen. Part of a section is shown in the accompanying photomicrograph.

SUMMARY

1. Rats on an adequate basal diet fed the residue left from cholesterol heated to 300° C. after the removal of dicholesteryl ether and Δ4-cholestenone, at

[1] The author is greatly indebted to Dr. John A. Mair, of the Chemistry Department, Glasgow University, for the quantitative analyses.

a level of 20 mgm. daily for 2 years, showed no tumor of the forestomach nor of the glandular zone.

2. Other rats fed Δ3,5-cholestadiene at a level of 25 mgm. daily for 2 years, plus an adequate basal diet, showed no tumor in either part of the stomach. It seems unlikely that this diene is concerned in the avitaminosis A induced by feeding heated fats to rats.

3. A large, inorganic bladder stone is reported in 1 rat of this series.

REFERENCES

1. Beck, S., and Peacock, P. R. Gastro-Papillomatosis Due to Vitamin A Deficiency Induced by Heated Fats. Brit. M. J., **2**:81-83. 1941.
2. Diels, O., and Linn, K. Zur Kenntnis des Cholesterins. Verein. Ber. dtsch. chem. Ges., **41**:260-266. 1908.
3. Eck, J. C., and Hollingsworth, E. W. A Study of Cholesterilene. Iowa State College J. Science, **13**:329-333. 1939.
4. Heilbron, I. M., and Sexton, W. A. Studies in the Sterol Group. Part II. The Formation of ψ-Cholestene and Cholestenone by the Dry Distillation of Cholesterol. J. Chem. Soc., 347-351. 1928.
5. Kirby, A. H. M. Attempts to Induce Stomach Tumors. I. The Effect of Cholesterol Heated to 300° C. Cancer Research, **3**:519-525. 1943.
6. Mauthner, J., and Suida, W. Beiträge zur Kenntniss des Cholesterins (III Abhandlung). Monatsh. Chem., **17**: 29-49. 1896.
7. Peacock, P. R., and Beck, S. Multiple Mesenteric Sarcomata in Rats Following the Ingestion of Heated Lard. Brit. J. Exper. Path. **24**:133-137. 1943.
8. Roffo, A. H. Acción cancerígena de los derivados fenantrénicos del colesterol. Bol. Inst. de med. exper. para el estud. y trat. d. cancer, **15**:837-845. 1938.
9. Roffo, A. H. Tumeurs malignes développées dans l'appareil digestif par l'ingestion de graisses oxydées par chauffage. Bull. Assoc. franç. p. l'étude du cancer, **28**: 556-588. 1939.
10. Roffo, A. H. Krebserzeugende Wirkung des aus dem Cholesterin gewonnenen Phenanthrenderivates. Ztschr. f. Krebsforsch., **49**:341-347. 1939.
11. Roffo, A. H. Pirolisis de colesterol alquitran cancerigeno del colesterol. Bol. Inst. de med. exper. par el estud. y trat. d. cancer, **18**:929-943. 1941.
12. Stavely, H. E., and Bergmann, W. The Chemistry of Unsaturated Steroids. I. The Constitution of Cholesterilene. J. Org. Chem., **1**:567-574. 1937.
13. Steiner, P. E., Steele, R., and Koch, F. C. The Possible Carcinogenicity of Overcooked Meats, Heated Cholesterol, Acrolein, and Heated Sesame Oil. Cancer Research, **3**: 100-107. 1943.
14. Strong, L. C. Seventh Scientific Report of The International Cancer Research Foundation, p. 17, 1939.
15. Thomson, W. Stock Diet for Rats. J. Hyg., **36**:24-25. 1936.
16. Veldstra, H. Δ3,5-Cholestadiene from Cholesteryl Oleate and Its Possible Bearing upon the Formation of Carcinogenic Substances in Heated Fats. Nature, London, **144**:246-247. 1939.
17. Wokes, F. Studies on Colour Tests for Sterols and Vitamin A. I. Sterol Tests. Biochem. J., **22**:830-835. 1928.

Experimental Brain Tumors

IV. The Incidence in Different Strains of Mice*

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Three chemical carcinogens, 20-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, have previously been tested for their brain tumor-inducing potentialities in C3H mice (6,7,2). Each chemical, in the form of a pellet of about 1.5 mm. diameter, was usually introduced into the subcortex of the right cerebral hemisphere, but in some instances the pellets were placed so deeply that they came to lie within the lateral ventricle. In the case of methylcholanthrene deliberate attempts were also made to place certain pellets both in the cerebellum and the cerebral leptomeninges. More recent experiments with this carcinogen (8) brought the total number of animals up to 145, of which 68 (47 per cent) developed primary brain tumors. Of these, 50 were gliomas and 18 were sarcomas. Forty-seven mice treated with benzpyrene yielded 24 (51 per cent) primary brain tumors—15 gliomas and 9 sarcomas. In contrast to the rather similar results obtained with these two chemical carcinogens, dibenzanthracene yielded but 4 brain tumors in 21 mice—2 gliomas and 2 sarcomas.

The purpose of the present communication is to report the results of intracerebral methylcholanthrene implantation in different strains of mice.

METHOD

Six strains of mice (C3H, ABC albino, Bagg albino, C57 black, A albino, and dba) were utilized in these experiments with 20-methylcholanthrene. The chemical carcinogen was obtained from the Edcan Laboratories, South Norwalk, Connecticut, and was employed in the form of pellets without purification or dilution. The pellets were made by heating the crystals gently in a beaker until fusion. When cooled, the solidified material was cut into 1 mm. cubes with a knife. Male and female mice in approximately equal numbers were used in each strain. The sexes were strictly segregated throughout the experiment. The mice were 6 to 7 weeks of age when the experiments were started.

All the details of the operative procedure, anesthesia, animal care, and diet were similar to that employed in the original work with methylcholanthrene (6). The right cerebral subcortex was the site of pellet implantation in about half the mice of each strain, whereas the cerebellum was the site in the other half of the animals. It should be recognized that the chemical was undoubtedly placed more deeply in the nervous tissue in some instances than in others and that there was no absolute means of avoiding the extrusion of certain pellets through the trephine opening in the skull into the tissues of the scalp.

RESULTS

Although availability was an important factor in the choice of the particular mouse strains employed in these experiments, deliberate selections were made in two instances. Those were in the cases of the C57 black and the dba strain. The former notoriously yields a low incidence of spontaneous breast tumors, whereas the latter is equally well known for its high incidence of this particular type of neoplasm. It seemed worth while to compare these two strains in their behavior to methylcholanthrene placed intracerebrally. The results of the experiments with all 6 mouse strains are summarized in Table I.

It is apparent from this table that two mouse strains, namely, the A albino and the dba, had a surprisingly low incidence of carcinogen-induced brain tumors. Since the very pellets of methylcholanthrene that failed to induce neoplasms in the mice of these two strains did later induce them in C3H mice, it appeared that the genetic constitution of the animals was the important factor determining tumor incidence. Burdette and Strong (4) showed similar variations in extracranial tumor susceptibility in five inbred strains of mice treated with subcutaneous injections of methylcholanthrene. They found that the C3H and JK strains were most and least susceptible, respectively, to such tumors. Further data on the genetic influence on carcinogen-induced brain tumors will be presented below.

* This investigation was aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

In nearly all the instances of extracranial sarcoma the pellets were found extruded into the tissues of the scalp. Similarly, the majority of the cases of intracranial sarcoma disclosed that the pellets were in contact with the meninges.

The types of glioma encountered in the different strains of mice are listed in Table II. Included in this table are also the classifications of the gliogenous portions of the neoplasms of mixed glioma-sarcoma type. To some extent the kind of glioma was determined by the site of carcinogen implantation. Thus, in the case of the ependymoblastomas, the pellets were in contact with the ventricular ependyma, whereas the medulloblastomas generally resulted from cerebellar implantations. This has already been discussed in a previous paper (6). The one mouse of the ABC albino strain (No. 5) and one of the three A albino mice (No. 8) that developed medulloblastomas had these tumors in the cerebrum. The reason for this unusual site of medulloblastoma is as obscure as the factors that determine why subcortical pellet implantation in the cerebrum results on occasion in astrocytoma, glioblastoma multiforme, or oligodendroglioma.

A rather disappointingly large number of gliogenous neoplasms could not be classified. Formerly it often proved possible to classify such tumors by studying their growth behavior and structural characteristics in transplants. In the present experiments, however, an opportunity to transplant such a tumor occurred but once, in a C3H mouse. The tumor was a mixed glioma-sarcoma, but the gliogenous portion was apparently lost in the very first transplantation.

Further data on the genetic influence on carcinogen-induced brain tumors.—Attention was called above to the fact that the incidence of methylcholanthrene-induced brain tumors was low in the dba in comparison with the C3H mice. Both strains of mice employed in these experiments were raised in this laboratory by pen matings—several females and one male in the same cage—from stock originally obtained in 1938 from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. It seemed worth while to repeat the intracerebral implantation of methylcholanthrene in inbred mice of these two strains obtained by brother-to-sister matings. Dr. Leonell C. Strong, of the Department of Anatomy, Yale University School of Medicine, supplied such inbred C3H mice and Doctor William S. Murray, of the New York State Institute for the Study of Malignant Diseases in Springville, New York, supplied the inbred dba mice. The origin of the various strains of mice employed in these experiments is fully described in a recent paper by Strong (5). To complete the study of the genetic influence on chemically induced brain tumors, hybrids were raised in this laboratory by crossing female pen-bred C3H mice with male pen-bred dba and also male pen-bred C3H with female pen-bred dba mice. In all these experiments the carcinogen was implanted in the right cerebral subcortex and the results are summarized in Table III.

In view of the 47 per cent incidence of intracranial neoplasms that resulted from methylcholanthrene implantation in pen-bred C3H mice, an incidence of 7 such tumors out of 27 Strong inbred C3H mice is

TABLE I: EFFECT OF METHYLCHOLANTHRENE IN DIFFERENT STRAINS OF MICE

Strain	No. of mice	Tumors	Negative	Gliomas	Intracranial sarcomas	Mixed glioma-sarcoma	Extracranial sarcomas	Unclassified tumors
C3H	42	34	8	10	10	5	9	—
ABC albino	25	19	6	14	3	—	2	—
Bagg albino	21	14	7	10	2	—	—	2
C57 black	35	21	14	10	4	—	7	—
A albino	22	6	16	4	—	—	2	—
dba	19	4	15	2	—	—	2	—

TABLE II: GLIOMAS IN DIFFERENT STRAINS OF MICE

	C3H	ABC albino	Bagg albino	C57 black	A albino	dba
Astrocytoma	1	—	1	1	—	—
Ependymoblastoma	2	4	3	—	—	1
Glioblastoma multiforme	2	8 *	3	1	—	1
Medulloblastoma	3	1	1	3	3	—
Oligodendroglioma	—	—	1 †	1	—	—
Unclassified	7	1	1	4	1	—

* One tumor was a mixed glioblastoma multiforme–medulloblastoma.
† This tumor was a mixed glioblastoma multiforme–oligodendroglioma.

disappointingly low. The anticipation, not realized in this experiment, was that an even greater percentage of primary brain tumors would be found in the inbred than in the pen-bred animals. The rather large number, 8, of extracranial sarcomas in this experiment was also unexpected. Perhaps, however, the number of mice employed, 27, is too small to justify the assumption that highly inbred C3H mice are refractory to carcinogen-induced brain tumors as compared to pen-bred animals of the same strain. It should be remembered in this connection that the C3H strain was developed entirely for its spontaneous breast tumor propensities and that its high yield of chemically-induced brain tumors is coincidental.

The results with the "Murray" inbred dba mice were as gratifying as those with the inbred C3H animals were disappointing. Of 29 mice in the experiment but one animal developed an intracranial tumor—a glioma. Equally suggestive of the genetic influence on carcinogen-induced brain tumors are the results with the F_1 progeny of C3H male and dba female, and C3H female and dba male animals. Of 46 mice in the former experiment, 13 developed primary brain tumors, and of 50 mice in the latter experiment, 12 had such tumors. Thus, whereas the pen-bred C3H mice yielded brain tumors in approximately 50 per cent of the animals and similarly bred dba mice yielded practically none, the hybrid offspring of these two strains had a tumor incidence of 25 per cent. Andervont (1) has already shown that C3H mice mated with I and Y mice produced a progeny whose susceptibility to induced tumors was intermediate to that of both parents. He concluded that if genetic factors were responsible for this susceptibility they were probably multiple. More recently, Burdette (3) reported that the progeny of C3H and JK mice had average and median appearance times for tumors induced by methylcholanthrene intermediate to those of the parental strains. He felt that his results were compatible with the existence of more than one gene for susceptibility to induced tumors. In so far as the incidence of carcinogen-induced brain tumors was also intermediate to that of the parental C3H and dba mice, the same conclusion is probably warranted; namely, that more than one genetic factor is responsible for the inheritance of susceptibility to such tumors.

A classification of the gliogenous neoplasms that developed in the inbred C3H and dba mice and in the F_1 hybrids from these two strains is presented in Table IV. The nongliogenous intracranial tumors were all fibrosarcomas with origins, as far as could be determined, in the cerebral meninges. The classifiable gliomas were of three varieties—astrocytoma, ependymoblastoma, and glioblastoma. Since the carcinogen was invariably implanted in the cerebral subcortex, at least a partial explanation is at hand for the failure of medulloblastomas to make their appearance. The relatively small total number of gliomas, 22, is probably another explanation for the relatively few varieties of tumors of this class to develop.

In the previous contributions to the general subject of experimental brain tumors already referred to (6, 7, 2) the different types of primary intracranial neoplasms were described and illustrated in detail as regards their macroscopic and histologic features. For this reason illustrative material is not included in the present report.

TABLE III: INCIDENCE OF BRAIN TUMORS IN INBRED AND HYBRID C3H AND DBA MICE

Strain	No. mice	Tumors	Negative	Gliomas	Intracranial sarcomas	Extracranial sarcomas
C3H (Strong)	27	15	12	4	3	8
dba (Murray)	29	1	28	1	—	—
C3H♂ × dba♀	46	21	25	7	6	8
C3H♀ × dba♂	50	20	30	10	2	8

TABLE IV: GLIOMAS IN INBRED AND HYBRID C3H AND DBA MICE

	Strong C3H	Murray dba	F_1 C3H♂ × dba♀	F_1 C3H♀ × dba♂
Astrocytoma	1	—	—	2
Ependymoblastoma	2	—	3	3
Glioblastoma multiforme	—	—	2	3
Unclassified	1	1	2	2

SUMMARY

The experiments comprising the subject of this communication were devised to test the validity of the hypothesis that genetic constitution is an important factor in the incidence of primary intracranial neoplasms induced with pure methylcholanthrene implanted intracerebrally. Of six strains of mice tested, namely, C3H, ABC albino, Bagg albino, C57 black,

A albino, and dba, the first four strains yielded an incidence of 50 per cent or better in primary intracranial neoplasms, whereas the last two strains were poor with respect to carcinogen-induced brain tumors. Only 4 of 22 A albino mice and 2 of 19 dba mice developed intracranial neoplasms. The incidence of brain tumors induced with methylcholanthrene is in no way related to the propensities that the different mouse strains show in regard to the development of spontaneous mammary tumors. Both the C3H and dba strains, for example, have a high incidence of spontaneous tumors of this variety, whereas the C57 black strain yields extremely few such tumors.

Of 29 inbred dba mice (from brother-to-sister matings) only 1 developed an intracranial tumor following the intracerebral implantation of methylcholanthrene. A disappointing fact was that only 7 of 27 similarly inbred C3H mice developed primary brain tumors, in contrast to an incidence of 25 brain tumors out of 42 pen-bred mice of the same strain. Hybrids obtained by crossing pen-bred C3H and pen-bred dba mice yielded an intracranial tumor incidence of 25 out of 96 animals, or 26 per cent.

REFERENCES

1. Andervont, H. B. The Incidence of Induced Subcutaneous and Pulmonary Tumors and Spontaneous Mammary Tumors in Hybrid Mice. Pub. Health Rep., **53**:1665-1671. 1938.
2. Arnold, H., and Zimmerman, H. M. Experimental Brain Tumors. III. Tumors Produced with Dibenzanthracene. Cancer Research, **3**:682-685. 1943.
3. Burdette, W. J. The Inheritance of Susceptibility to Tumors Induced in Mice. II. Tumors Induced by Methylcholanthrene in the Progeny of C3H and JK Mice. Cancer Research, **3**:318-320. 1943.
4. Burdette, W. J., and Strong, L. C. The Inheritance of Susceptibility to Tumors Induced in Mice. I. Tumors Induced by Methylcholanthrene in Five Inbred Strains of Mice. Cancer Research, **3**:13-20. 1943.
5. Strong, L. C. The Origin of Some Inbred Mice. Cancer Research, **2**:531-539. 1942.
6. Zimmerman, H. M., and Arnold, H. Experimental Brain Tumors. I. Tumors Produced with Methylcholanthrene. Cancer Research, **1**:919-938. 1941.
7. Zimmerman, H. M., and Arnold, H. Experimental Brain Tumors. II. Tumors Produced with Benzpyrene. Am. J. Path., **19**:939-955. 1943.
8. Zimmerman, H. M., and Arnold, H. Chemical Carcinogens and Animal Species as Factors in Experimental Brain Tumors. J. Neuropath. and Exper. Neurol., **2**:416-417. 1943.

Factors That Alter the Fluorescence of Certain Carcinogens*

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Under the same title Miller and Baumann (3) have recently reported results that have many points in common with unpublished observations made some time ago in this laboratory. In so far as these supplement the experiments of Miller and Baumann, they may be of general interest.

Effects of Solvents

Table I shows the relative fluorescence intensities of benzpyrene solutions obtained with a series of hydrocarbons and a series of alcohols as solvents. It will be noticed that in both classes there are solvents in which the fluorescence is at least as high as in the ethers mentioned by Miller and Baumann. Similar differences were found with a series of esters. There is thus no reason to assume any special virtue of ethers as a group.

Table I: Relative Fluorescence Intensities of 3,4-Benzpyrene Solutions

Concentration: 5 μgm./5 ml.

Solvents	Galvanometer readings (Arbitrary units)
(1) Hydrocarbons:	
Hexane	74
Cyclohexane	154
Toluene	163
Xylene	169
Benzene	191
Dekalin	254
Pinene	262
Tetralin	330
Paraffin oil	444
(2) Alcohols:	
Methanol	136
Ethanol	160
n-Propanol	198
Isobutanol	211
n-Butanol	215
Amyl alcohol	239
Cyclohexanol	357
Glycol	362
Glycerol	414

Attention has previously been drawn to these differences in fluorescence intensity according to the solvent used (6); they were explained by the different solubility of O_2 in these solvents and by a quenching effect of the dissolved O_2 on the fluorescence. The differences observed in O_2, and to a lesser degree in air, are greatly reduced if the measurements are carried out in N_2 (Table II). This applies particularly to the effect of addition of water to a solvent. Miller and Baumann state "that difference in oxygen solubility was not the sole cause of the observed change in fluorescence." Without in any way contradicting this view in general I think that the argument of these authors, which is based on a curve showing the fluorescence changes of benzpyrene solutions in ethanol-water mixtures, is not convincing. Miller and Baumann say that "at the extremes of the concentration range the rates of change of O_2 solubility with percentage of solvent were either too large or too small in comparison with the corresponding changes in fluorescence." It may be doubted, however, whether fluorescence measurements in an ethanol-water mixture of less than 30 per cent ethanol may be safely compared with those of higher ethanol content owing to the rapidly decreasing solubility of benzpyrene with increasing percentage of water; the decrease of fluorescence observed in this part of the curve may in fact be due to physical changes in the dispersion of the solute. The decrease of fluorescence at the other extreme of the concentration range does not seem to be smaller than might be expected if it is borne in mind that the fluorescence yield in the presence of O_2 does not decrease linearly with the O_2 concentration, but can be represented by a hyperbolic curve (1). The measurement of fluorescence in the absence of O_2 provides more direct evidence, and shows that the differences in the fluorescence of benzpyrene solutions in 100 per cent and 60 per cent ethanol observed in air practically disappear. This, however, is not generally true for all hydrocarbons or for all solvents, and it must be admitted that a

* Because of the difficulties of international communication the author has not read proof of this article.

† Some of the experiments reported in this paper were carried out in collaboration with Dr. J. Weiss, Department of Chemistry, King's College, Newcastle upon Tyne.

solvent effect in the proper sense persists even in N_2 in many cases (Table II).

Table II: Relative Fluorescence Intensities of Hydrocarbon Solution in O_2, Air, and N_2

Hydrocarbon	μgm./5 ml.	Solvent	Galvanometer readings (Arbitrary units) In O_2	In air	In N_2
3,4-Benzpyrene	10	Hexane	8	63	410
		Benzene	50	175	445
		Ethanol	24	135	403
		60% Ethanol	173	295	405
		Acetic acid	43	140	355
		80% Acetic acid	148	268	358
Anthracene	50	Hexane	15	60	95
		Benzene	52	100	108
		Ethanol	34	76	88
1,2-Benzanthracene	50	Hexane	ca. 1	11	52
		Benzene	5	53	151
		Ethanol	ca. 1	26	75
20-Methylcholanthrene	10	Hexane	ca. 1	11	72
		Benzene	20	80	143
		Ethanol	8	40	91
1,2,5,6-Dibenzanthracene	30	Hexane	ca. 1	5	13
		Benzene	ca. 1	9	20
		Ethanol	ca. 1	6	14

Table III: Stability of Fluorescence of 3,4-Benzpyrene Solutions (10 μgm./5 ml.) During 20 Minutes' Ultraviolet Irradiation in O_2 and in N_2

Solvent	Gas	Galvanometer reading 0 min.	5 min.	10 min.	15 min.	20 min.	% of initial reading 5 min.	10 min.	15 min.	20 min.
Hexane	N_2	405	410	410	410	415	101	101	101	102
	O_2	8	8	8	8	8	100	100	100	100
Benzene	N_2	438	445	445	448	450	102	102	102	103
	O_2	50	50	50	50	50	100	100	100	100
Ethanol	N_2	403	392	378	363	349	97	94	90	87
	O_2	24	23	22.5	22	22	96	94	92	92
60% Ethanol	N_2	405	384	356	328	299	95	88	81	74
	O_2	173	164	162	159	157	95	94	92	91
60% Ethanol + 40% N/10 HCl	N_2	395	370	360	340	328	94	91	86	83
	O_2	173	170	160	155	150	98	93	90	87
60% Ethanol + 40% N/10 NaOH	N_2	390	328	275	238	200	84	70	61	51
	O_2	175	170	160	155	148	97	92	89	85
Acetic acid	N_2	355	343	323	316	298	97	93	89	84
	O_2	43	35	32	29	26	83	75	67	60
80% Acetic acid	N_2	358	328	312	300	288	92	87	84	81
	O_2	148	97	63	44	25	66	42.5	30	17
		0 min.	1 min.	2 min.	3 min.	4 min.	1 min.	2 min.	3 min.	4 min.
Chloroform	N_2	250	90	41	20	16	30	13.5	6.7	5.3
	Air	130	75	48	32	23	52	33	22	16

Stability of Fluorescence Intensity

This factor, too, depends to a large extent on the solvent used. The fluorescence of a benzpyrene solution in hexane or benzene (2 μgm./ml.) does not change appreciably during 20 minutes' irradiation (Table III). There is, however, a slight falling off in the more polar solvents ethanol and acetic acid. In the latter solvent the presence of O_2 enhances the fall of fluorescence. This effect of O_2 becomes very striking indeed in a mixture of 80 per cent acetic acid and 20 per cent water: here the fluorescence decreases to 17 per cent of the initial value in 20 minutes, as compared with 81 per cent in N_2. Simultaneously with the destruction of fluorescence a yellow tinge appears in the solution. It is probable that the changes

observed are due to a very rapid photooxidation. Addition of water to ethanol increases the instability of the fluorescence only slightly. In this case O_2 has, if anything, a stabilizing influence that is probably connected with its quenching effect on fluorescence. The rapid photooxidation observed in 80 per cent acetic acid suggested a possible influence of hydrogen ions and the effect of pH variation in ethanol-water mixtures was therefore investigated. No photooxidation was observed in either acid or alkaline medium; this makes it unlikely that hydrogen ions are concerned in the effect of 80 per cent acetic acid. On the other hand, the nonoxidative destruction of the hydrocarbon was significantly accelerated in an alkaline medium.

A very rapid destruction of the fluorescence of benzpyrene takes place in chloroform solution, as has been found by Miller and Baumann also. This reaction is also nonoxidative.

Inhibitors of Fluorescence

Amongst the inhibitors of fluorescence in solution three categories can be distinguished:

(a) Substances having a genuine quenching effect due to a *reversible* photochemical reaction between the fluorescent and the inhibitor substances, a reaction that is usually in the nature of a reversible oxidation-reduction system. The best known examples are: the action of I^- on uranium salts (2), the action of Fe^{++} on methylene blue and similar dyes (8), the action of O_2 (1, 6) and of NO (7) on hydrocarbons.

(b) Inhibition of fluorescence due to *irreversible* reactions, which may be either photochemical, as is probably the case when benzpyrene is irradiated in halogenated solvents, or of the ordinary thermal type. The effect of tetranitromethane, quoted by Miller and Baumann, seems to belong to this class. A similar irreversible "inhibition" of benzpyrene fluorescence can be achieved with numerous other reagents, *e.g.,* by shaking with a drop of concentrated HNO_3 or H_2SO_4 or by the addition of strong oxidants.

(c) Filter effects: many substances prevent fluorescence owing to the fact that they strongly absorb in the region of the exciting wave lengths. This point can be tested with a vessel of the type illustrated in Fig. 1. The fluorescent solution is placed in the inner tube and a solution of the inhibitor in the jacket. If the inhibition of fluorescence is due to light absorption it will be equal to that observed if the inhibitor is added directly to the fluorescent solution. In this way it can be shown that the absence of fluorescence of benzpyrene solutions in solvents such as CS_2, benzaldehyde, quinoline, nitrobenzene, and others is primarily a filter effect. Nitro compounds generally belong to this class; nitrobenzene, trinitrobenzene, and picric acid were tested and were found to act in very low concentrations. In addition to its irreversible action on benzpyrene tetranitromethane may be expected to have a strong filter effect, which explains its general action in suppressing fluorescence.

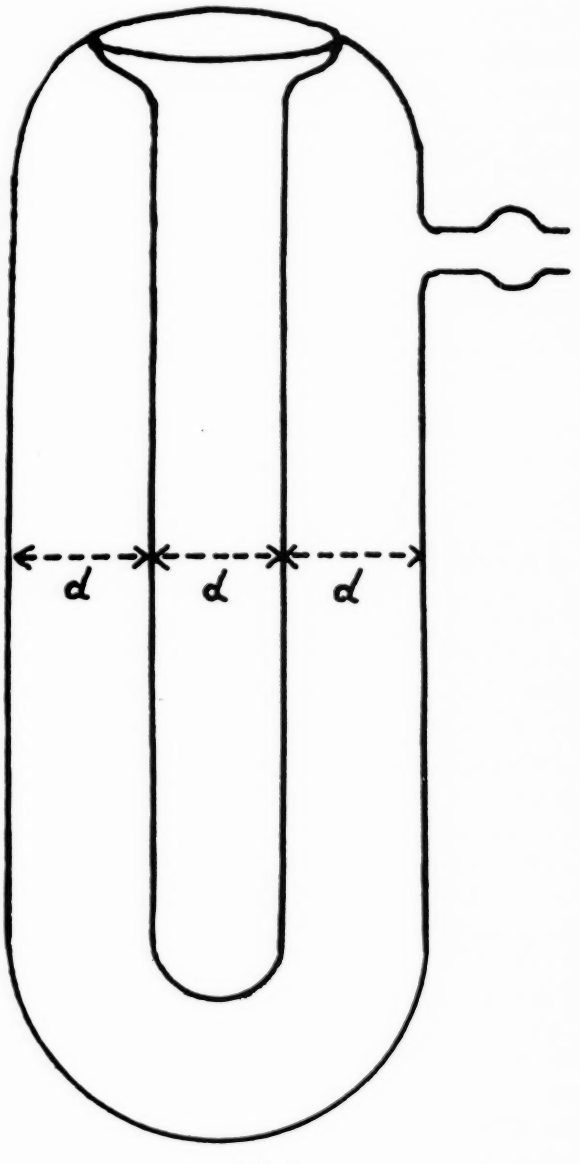

Fig. 1

A filter effect is always to be suspected if the inhibitor has a yellow color. Such inhibitors are, *e.g.,* carotene, *p*-dimethylaminoazobenzene and other azo dyes, and the yellow polymerization products of acetaldehyde that are formed if impure alcohol is heated with alkali.

The vessel shown in Fig. 1 has also been used to demonstrate that the quenching effect of O_2 and of nitric oxide is not a filter effect. For this purpose it was filled with pure solvent to a height just above

the level of the fluorescent solution in the inner tube. The jacket was then evacuated and filled with the gas to be examined.

Fluorescence of Animal Material

Miller and Baumann have found that the fluorescence of unsaponifiable animal material is less dependent on the solvent than that of hydrocarbons. This is due to the fact that it is less susceptible to the quenching effect of O_2. Whereas the fluorescence of benzpyrene in benzene solution is increased by about 150 per cent if the measurement is carried out in N_2 as compared with air, that of the unsaponifiable matter of a whole mouse, or rather its small residue that cannot be separated from benzpyrene by chromatographic purification, is increased by only about 80 per cent. For this and other reasons, *viz.*, the higher intensity of fluorescence and the greater independence from solvent effects, it has been the practice in this laboratory to carry out all fluorimetric determinations of hydrocarbons in N_2. Details of the method used are shortly to be published (4).

EXPERIMENTAL

The procedure and apparatus used for the fluorimetric estimation of 3,4-benzpyrene have been described (5). For the experiments reported in this paper the same apparatus was used with slight modifications: a more sensitive photocell (EEL selenium barrier layer cell) of 45 mm. diameter was used and the fluorescent light was filtered, in addition to the layer of 5 per cent sodium nitrite, through two Wratten filters, Nos. 35 + 43, with a maximum transmission at about 400 mμ. Saturation of the solution with O_2 or with oxygen-free N_2 was usually carried out in the dark for 2 to 3 minutes. In the experiments of Table III gassing was continued during the intervals between readings with the solution in the beam of the ultraviolet light. The gas stream was saturated with the solvent used by passage through a wash bottle before it entered the fluorescent solution.

A zero reading with pure solvent was taken in every case and all figures have been corrected accordingly.

Sensitivity of galvanometer deflection was controlled by inserting shutters of varying aperture between light source and solution. The results of Table I are thus recorded on a scale different from that used for Tables II and III.

SUMMARY

Observations are reported and discussed with reference to the experiments of Miller and Baumann (3). The following points are made:

1. The chemical classification of a solvent has no relation to the fluorescence intensity of hydrocarbons dissolved in it.

2. For the particular case of 3,4-benzpyrene solution in ethanol-water mixtures it can be shown that the differences in fluorescence intensity according to the percentage of solvent composition are entirely due to a quenching effect of dissolved O_2. The differences disappear in N_2. In many other cases the solvent effect on fluorescence intensity can be largely accounted for by the quenching effect of O_2, though there are exceptions where a solvent effect proper persists even in the absence of O_2.

3. The stability of the fluorescence of benzpyrene solutions also depends on the solvent. No appreciable change occurs in hexane or benzene during a 20 minute irradiation. In acetic acid, and especially in 80 per cent acetic acid—20 per cent water mixture, photooxidation takes place in presence of O_2. In ethanol and in mixtures of 60 per cent ethanol with 40 per cent $N/10$ HCl, water or $N/10$ NaOH a fairly slow nonoxidative fall of fluorescence, increasing in the order named, is observed.

The rapid destruction of fluorescence in chloroform is confirmed and is shown to be nonoxidative reaction.

4. Inhibitors of fluorescence in solution belong to one of three categories: (a) substances causing a *reversible* photochemical reaction ("genuine quenching"); (b) substances causing *irreversible* changes of the fluorescent material; (c) substances that absorb the exciting wave lengths ("filter effect"). Many solvents in which hydrocarbons do not fluoresce belong to this class.

5. An unsaponifiable fraction of mouse tissues is less susceptible to the quenching effect of O_2 than carcinogenic hydrocarbons. It is recommended to carry out fluorimetric determinations of hydrocarbons in N_2.

REFERENCES

1. Bowen, E. J., and Williams, A. H. The Photo-Oxidation of Hydrocarbon Solutions. Tr. Faraday Soc., **35**:765-771. 1939.
2. Carter, A. H., and Weiss, J. The Transfer of Excitation Energy from Uranium Ions in Solution. Proc. Roy. Soc., A, **174**:351-370. 1940.
3. Miller, J. A., and Baumann, C. A. Factors That Alter the Fluorescence of Certain Carcinogens. Cancer Research, **3**:223-229. 1943.
4. Weil-Malherbe, H. Nineteenth Annual Report, British Empire Cancer Campaign, 1942, p. 41.
5. Weil-Malherbe, H. An Experimental Study of Chromatography. J. Chem. Soc., 1943, p. 303.
6. Weil-Malherbe, H., and Weiss, J. Reversible Quenching by Oxygen of the Fluorescence of Polycyclic Hydrocarbons. Nature, London, **149**:471-472. 1942.
7. Weil-Malherbe, H., and Weiss, J. Quenching of Fluorescence by Nitric Oxide. Nature, London, **151**:449. 1943.
8. Weiss, J. Photosensitised Reactions and the Quenching of Fluorescence in Solution. Tr. Faraday Soc., **35**:48-55. 1939.

Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture

II. The Delayed Lethal Effect*

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In a previous communication (4) we showed that the "immediate lethal dose" of x-rays for rat sarcoma cells is the same as that for normal rat fibroblasts. Doses of 200,000 r uniformly and immediately check all cell outwandering in cultures of normal as well as neoplastic cells. In continuation of our comparative studies on the radiosensitivity of normal and malignant cells, we undertook experiments to determine the "delayed lethal dose" of x-rays for sarcoma cells cultivated *in vitro, i.e.,* the dose that produces an irreversible injury to the irradiated cell cultures causing the death of their descendants in the course of successive passages. This dose has been compared with the corresponding dose for normal cells.

MATERIAL AND PROCEDURE

Cell cultures.—The experiments were performed on standardized cultures of chicken fibroblasts derived from the hearts of 7 day old embryos, and on cultures of benzpyrene-induced rat sarcoma. The sarcoma cell strain has to date been kept in this laboratory without loss of malignancy for over 2 years. The cell colonies were cultivated in hanging drops according to the standard method of Carrel. As culture medium we used in both cases normal chicken plasma and diluted chicken embryo extract, in the proportion 1:1. Growth curves of the cultures were constructed from planimetric measurements of outline drawings of the surface areas made every 24 hours.

Radiological technic.—The technic of irradiation was as described in the first paper of this series (4).

Experimental procedure.—Cultures of sarcoma cells were irradiated, immediately after having been transferred, with the following x-ray doses: 500, 1,000, 1,500, 2,000, and 2,500 r. The irradiated cell colonies were cultivated throughout successive passages. Subcultures were made at intervals of 3 days, and transfer was continued as long as the cultures survived, or until full recovery had been attained.

FINDINGS

The results of our experiments may be summarized as follows: Irradiation with 500 r does not produce permanent damage of the sarcoma cell culture; with few exceptions cultures exposed to this dose survive, and after several passages show a quite normal rate of growth. Irradiation with 1,000 r produces variable results; out of 27 cultures treated with 1,000 r 12 succumbed in the course of the succeeding transfers, while 15 cultures survived. After a radiation with 1,500 r only 5 out of 37 could be cultivated continuously, the remainder dying after an average of 3 passages. A dose of 2,000 r rendered prolonged cultivation of the irradiated cultures uniformly impossible. This dose is, therefore, to be regarded as the minimal delayed lethal dose for rat sarcoma cells.

The curves plotted in Figs. 1 and 2 exemplify the difference in the growth rate of sarcoma cell colonies derived from cultures irradiated with 1,500 and 2,000 r respectively and carried through successive passages.

DISCUSSION

Studies on the effect of x-rays upon cells growing *in vitro* have shown that with sufficiently high x-ray doses the outgrowth of the irradiated cell culture can be checked immediately and completely (1). It is also possible to produce a lethal effect on cells *in vitro* by exposure to considerably smaller x-ray quantities (3). X-ray doses that are themselves too low to cause immediate cessation of growth of the irradiated culture are capable of exerting a lethal action after a latent period. Cultures treated with such x-ray doses continue to grow for some time, but after a certain number of passages they die ("delayed lethal effect").

There is a wide gap between the x-ray dose that produces an immediate lethal effect and that which produces a delayed lethal effect. For cultures of nor-

* Because of the difficulties of international communication the authors have not read proof of this article.

mal chicken fibroblasts, for example, the immediate lethal dose is 50 times greater than the delayed lethal dose (2). The great divergence between these two multiplication. Cultures irradiated with such a dose die in the course of subsequent transfers because the cells become incapable of normal division; the subcul-

TABLE I: EFFECT OF IRRADIATION ON THE CULTIVATABILITY OF RAT SARCOMA CELLS

Irradiation with 500 r

Experiment number	Results
22914	+
22926	+
22951	+
22955	+
22952	+
22957	—(4)
22962	+
22967	+
23966	+
Summary	8+; 1—

Irradiation with 1,000 r

Experiment number	Results
22907	—(6)
22908	+
22913	+
22919	—(6)
22920	+
22925	+
22990	+
22996	—(5)
23002	+
23008	—(4)
23014	+
23089	+
23091	+
23093	—(2)
23095	+
23268	+
23270	—(2)
23272	+
23774	+
23278	+
23383	—(3)
23887	—(3)
23905	—(3)
23907	—(4)
23967	+
23979	—(7)
23981	—(7)
Summary	15+; 12—

Irradiation with 1,500 r

Experiment number	Results
23146	+
23148	—(4)
23150	+
23269	—(3)
23271	—(2)
23273	+
23275	+
23277	—(3)
23279	+
23384	—(3)
23388	—(2)
23497	—(2)
23499	—(3)
23505	—(3)
23517	—(3)
23662	—(2)
23664	—(2)
23670	—(3)
23674	—(2)
23676	—(2)
23686	—(3)
23743	—(3)
23747	—(3)
23749	—(3)
23761	—(4)
23767	—(3)
23779	—(3)
23888	—(2)
23894	—(2)
23900	—(3)
23906	—(2)
23908	—(3)
24520	—(3)
24522	—(3)
24524	—(3)
24526	—(4)
24528	—(3)
Summary	5+; 32—

Irradiation with 2,000 r

Experiment number	Results
23147	—(3)
23149	—(3)
23151	—(3)
23498	—(3)
23500	—(4)
23506	—(4)
23518	—(4)
23520	—(4)
23663	—(2)
23665	—(2)
23671	—(3)
23675	—(2)
23677	—(2)
23687	—(2)
23744	—(3)
23748	—(3)
23750	—(3)
23762	—(3)
23764	—(2)
23768	—(3)
23774	—(3)
24519	—(3)
24521	—(2)
24523	—(3)
24525	—(3)
24527	—(3)
24529	—(3)
Summary	0+; 27—

Irradiation with 2,500 r

Experiment number	Results
22671	—(2)
22672	—(2)
22683	—(2)
22685	—(2)
22686	—(2)
22991	—(3)
22997	—(3)
23003	—(3)
23009	—(3)
23013	—(3)
23080	—(2)
23092	—(5)
23094	—(2)
23096	—(2)
Summary	0+; 14—

+ Prolonged cultivation possible.
— Prolonged cultivation impossible.
Bracketed figures represent the number of passages through which cultivation of the irradiated cultures could be carried.

doses is due to the fact that the mechanism of their action is fundamentally different. The delayed lethal dose affects a particularly susceptible cell function— tivation of these cultures is therefore possible only so long as the original cell reserve is not exhausted. The immediate lethal dose, on the other hand, affects not

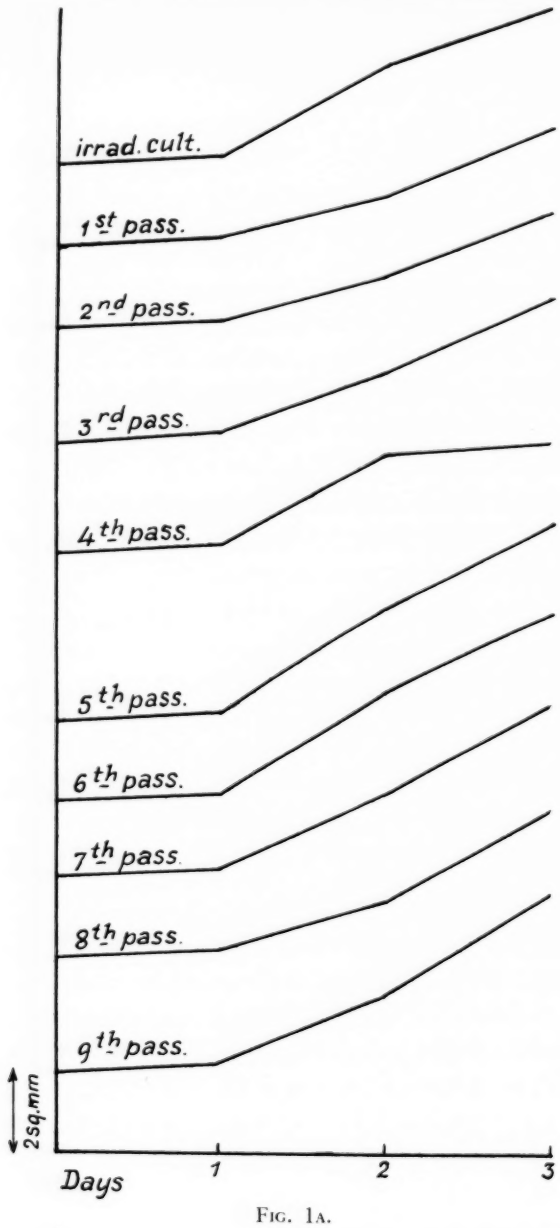

FIG. 1A.

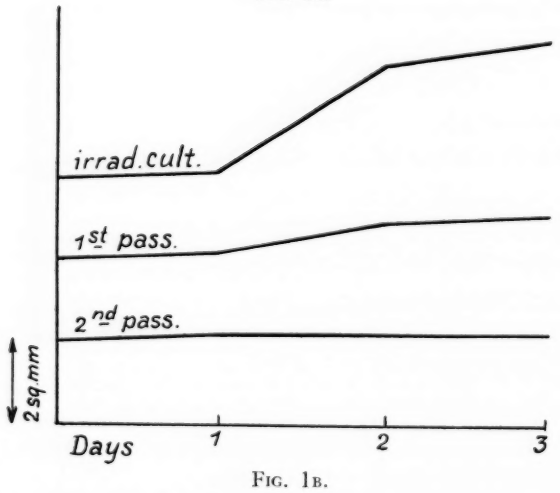

FIG. 1B.

FIG. 1.—Exp. No. 23146/7. Growth rate of sarcoma cell colonies derived from sarcoma cultures (sister halves) irradiated with 1,500 r (A) and with 2,000 r (B).

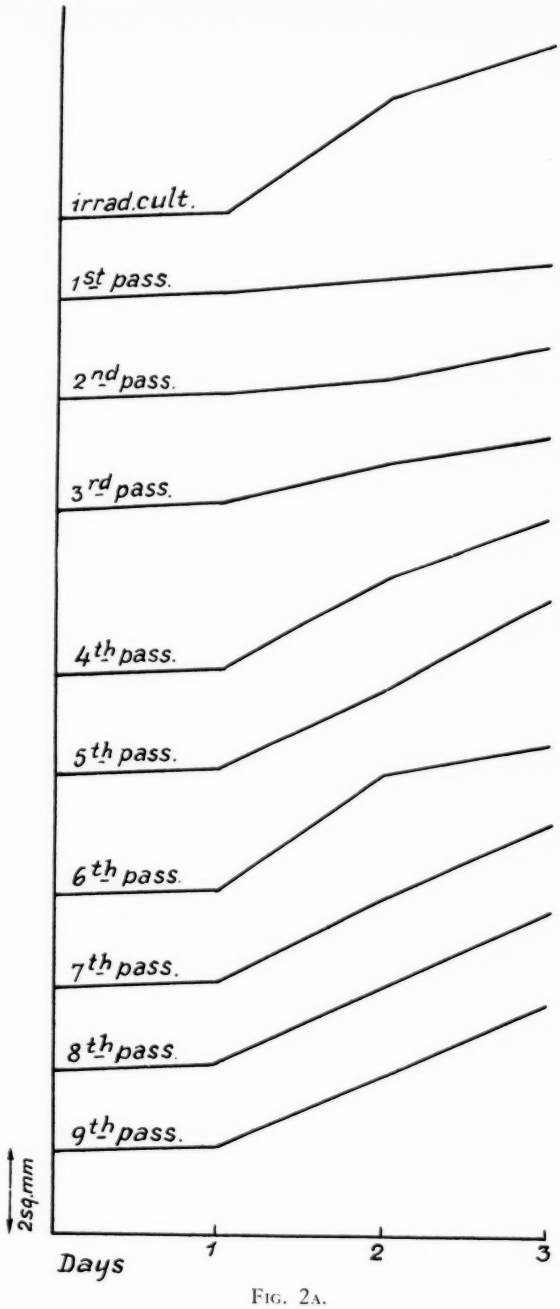

FIG. 2A.

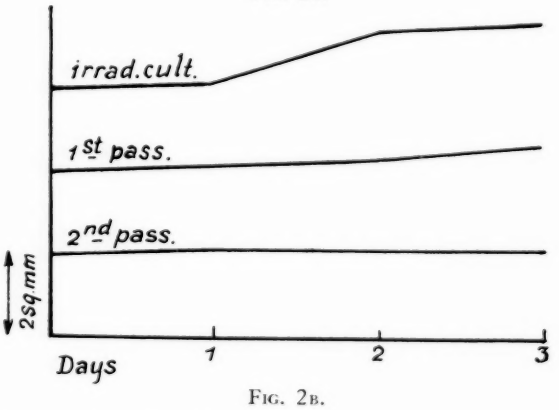

FIG. 2B.

FIG. 2.—Exp. No. 23245/151. Growth rate of sarcoma cell colonies derived from sarcoma cultures irradiated with 1,500 r (A) and with 2,000 r (B).

only the mitotic but also other fundamental cell funtions, especially cell motility; the effect of this dose is perceptible at once after irradiation and is manifested in the cessation of all outgrowth in the irradiated culture itself.

As we have shown in the previous paper (4), there is no difference as to the dose producing an immediately lethal effect between normal and sarcoma cells respectively; a dose of 200,000 r arrests kinetic activity and immediately stops outwandering in both types of cells, the malignant as well as the normal. In continuation of these experiments we attempted to determine the relative susceptibility to x-rays of the mitotic activity of normal and malignant cells. For this purpose the dose that interferes with prolonged cultivation of rat sarcoma cells, delayed lethal dose, has been evaluated and will be compared with corresponding doses for normal cells.

The experiments reported in the present paper have demonstrated that the lowest x-ray dose that renders rat sarcoma cells incapable of prolonged cultivation, the delayed lethal dose, is 2,000 r. Comparison reveals that this dose is far smaller than that which causes the same effect in normal chicken fibroblasts. Irradiation with 2,000 r and even with doses 50 per cent higher (3,000 r) produces in normal chicken fibroblasts a transient decrease in the growth rate, which is completely overcome after 2 or 3 passages. Permanent cultivation of normal chicken fibroblasts is prevented only when a dose of 5,000 r, *i.e.,* more than twice the delayed lethal dose for rat sarcoma cells, is applied (3, 2).

The significance of these findings is restricted, comparison of the radiosensitivity of normal and malignant cells having been drawn between cells of different species. Technical difficulties in cultivating permanent strains of normal rat fibroblasts, as was originally intended, rendered their use as a standard of comparison in the present study impracticable.

SUMMARY

The delayed lethal dose of x-rays for rat sarcoma cells cultivated *in vitro* is 2,000 r. This is less than half the equivalent dose for chicken normal fibroblasts, whose proliferative capacity is irreversibly checked only after irradiation with 5,000 r.

REFERENCES

1. Doljanski, L., and Goldhaber, G. Radiobiological Studies on Tissue Cultures. I. The Immediate Effect of X-Rays on Cell Outgrowth in Cultures of Fibroblasts. Growth, **6**:235-249. 1942.
2. Doljanski, L., and Goldhaber, G. Delayed Lethal Effect of X-Rays on Fibroblasts Cultivated *in vitro*. In press.
3. Goldhaber, G., and Doljanski, L. Radiobiological Studies on Tissue Cultures. II. The Delayed Effect of X-Rays on Cell Outgrowth in Cultures of Fibroblasts. Growth, **6**: 357-366. 1942.
4. Halberstaedter, L., Goldhaber, G., and Doljanski, L. Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture. I. The Effect of X-Rays on Cell Outgrowth in Cultures of Normal Rat Fibroblasts and Rat Benzpyrene-Induced Sarcoma. Cancer Research, **2**:28-31. 1942.

Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture

III. Further Studies on the Inhibitory Effect of X-Rays on Cell Outgrowth*

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In our comparative studies on the effect of x-rays on normal and malignant cells cultivated *in vitro* it was shown: (a) that the "immediate lethal dose" of x-rays is the same for rat sarcoma cells as for rat normal fibroblasts (3); (b) that the minimum dose producing a "delayed lethal effect" is appreciably smaller for rat sarcoma cells than for chicken normal fibroblasts (2).

The last finding suggested that the proliferative capacity of rat sarcoma cells *in vitro* is particularly susceptible to x-rays. In continuation of these studies it seemed of interest to determine the minimal x-ray dose that causes a measurable inhibition of growth rate in an irradiated sarcoma culture and to compare this dose with that which evokes a corresponding effect in normal cells. Assuming that proliferative capacity in sarcoma cells is particularly susceptible to x-rays, it was to be expected that it would be possible to inhibit outgrowth in an irradiated sarcoma culture by a lower x-ray dose than would be required to produce a corresponding effect in a culture of normal fibroblasts.

MATERIAL AND METHODS

Technic of cultivation and radiological technic were as described in the first papers of this series (3, 2).

Experimental procedure.—To test the effect of x-rays, the culture was divided into halves before transference to fresh medium, one serving as the control and the other for irradiation. The irradiation was carried out immediately after transference. After irradiation controls and experimental cultures were returned to the incubator. The growth rates of the control and irradiated cell colonies were recorded for a period of 3 days.

FINDINGS

The results of the present investigation may be summarized as follows: Irradiation of sarcoma cultures with 25,000, 10,000, and 5,000 r respectively causes 79 per cent, 69 per cent, and 52 per cent inhibition of the growth rate compared with the non-irradiated controls. Irradiation with 1,000 r produces a decline in outgrowth of 40 per cent. Five hundred r are also effective and cause a considerable decrease (23 per cent) in growth rate. Irradiation with 250 r is without significant effect on the outgrowth of the irradiated sarcoma cell colony.

DISCUSSION

It has been shown that 500 r of x-rays produces a definite inhibition of the outgrowth of an irradiated sarcoma cell colony. A dose of 250 r, on the other hand, is without significant effect.

The dose of 500 r that causes definite inhibition of growth of sarcoma cell cultures is considerably lower, as comparison shows, than the smallest dose evoking an equivalent effect in normal fibroblasts. In cultures of normal chicken fibroblasts, irradiation with 500 r is completely ineffective and even 1,000 r fail to produce definite retardation of outgrowth. To obtain a significant, if small, inhibition of growth rate in cultures of normal chicken fibroblasts, a dose of 2,500 r must be applied (1).

Attention must be directed to the fact that as the dose of x-rays is increased the difference in the response of the two cell types, normal and malignant, tends to diminish. At doses of 1,000 r and less the effect of irradiation on rat sarcoma cells and normal fibroblasts is notably different, but at doses of 5,000 r and more the difference becomes less distinct. Doses of 5,000, 10,000, and 25,000 r cause in cultures of rat sarcoma cells inhibition of 52 per cent, 69 per cent, and 79 per cent respectively, and in normal chicken fibroblasts inhibition of 42 per cent, 46 per cent, and 54 per cent respectively (1).

The curves in Fig. 1 illustrate the different reaction of normal and malignant cells to x-rays. A definite

* Because of the difficulties of international communication the authors have not read proof of this article.

TABLE I

	Experiment number	Size (mm²) of growth area after 72 hours of cultivation		
		Exp.	Control	Exp. /Control, per cent
Irradiation with 25,000 r	18366	1.25	3.8	33
	18370	1.1	3.55	31
	18378	1.45	3.35	41
	24610	0	4.75	0
	24700	0.5	5.45	9
	24716	0.7	5.0	14
	Mean			21
Irradiation with 10,000 r	18364	0.65	3.25	20
	18388	0.6	5.05	12
	24696	1.1	4.35	25
	24704	1.75	3.65	48
	24708	1.95	5.4	36
	24712	1.6	3.7	43
	Mean			31
Irradiation with 5,000 r	18543	3.0	4.4	68
	24588	2.45	5.1	48
	24614	2.6	4.75	55
	24694	1.75	4.9	36
	24702	2.2	5.35	41
	24714	2.3	5.7	40
	Mean			48
Irradiation with 1,000 r	24496	4.1	6.8	60
	24500	2.05	5.8	35
	24508	3.15	6.9	46
	24512	4.1	6.15	67
	24586	3.2	4.3	74
	24612	3.15	4.15	76
	Mean			60
Irradiation with 500 r	24385	3.65	5.65	65
	24466	5.95	7.4	80
	24474	5.1	5.5	93
	24494	4.9	4.75	103
	24498	2.9	4.05	72
	24510	2.85	5.25	54
	Mean			77
Irradiation with 250 r	24331	2.65	3.2	83
	24377	5.05	5.3	95
	24383	6.1	6.1	100
	24407	3.85	4.05	95
	24452	6.85	7.1	97
	24465	4.35	4.05	107
	Mean			94

inhibition of outgrowth is evident in sarcoma cell cultures at doses that are practically ineffective in normal cell cultures. As doses are increased the curves approach one another.

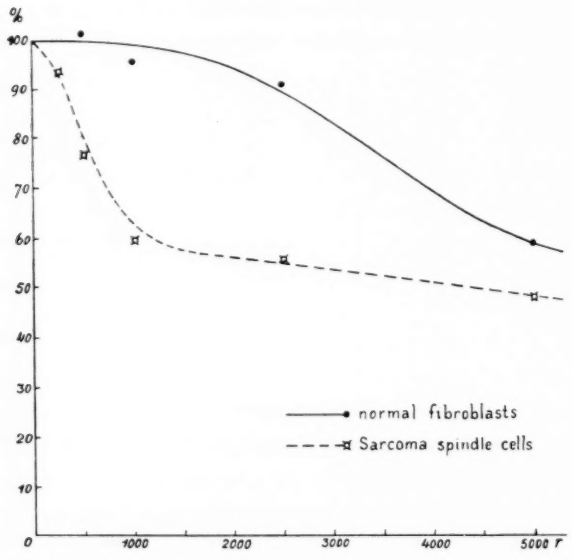

FIG. 1.—Effect of x-rays on outgrowth of normal chicken fibroblasts and rat sarcoma cells cultivated *in vitro*. *Abscissae*—X-ray dose in r. *Ordinates*—Outgrowth area of irradiated cultures relative to that of nonirradiated sister cultures in per cent.

The experimental evidence presented in this paper gives additional support to the assumption that the proliferative capacity of rat sarcoma cells is more susceptible to x-rays than that of normal fibroblasts. The limited significance of conclusions as to the comparative x-ray susceptibility of a given cell function, when they are made on the basis of comparisons between cells not of the same species, has already been pointed out in the preceding communication (2).

SUMMARY

The minimum dose of x-rays that causes appreciable inhibition of growth rate in an irradiated sarcoma cell culture is 500 r. The corresponding dose for normal chicken fibroblasts is 2,500 r.

REFERENCES

1. DOLJANSKI, L., and GOLDHABER, G. Radiobiological Studies on Tissue Cultures. I. The Immediate Effect of X-Rays on Cell Outgrowth in Cultures of Fibroblasts. Growth, **6**: 235-249. 1942.
2. DOLJANSKI, L., GOLDHABER, G., and HALBERSTAEDTER, L. Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture. II. The Delayed Lethal Effect. Cancer Research, **4**:106-109. 1944.
3. HALBERSTAEDTER, L., GOLDHABER, G., and DOLJANSKI, L. Comparative Studies on the Radiosensitivity of Normal and Malignant Cells in Culture. I. The Effect of X-Rays on Cell Outgrowth in Cultures of Normal Rat Fibroblasts and Rat Benzpyrene-Induced Sarcoma. Cancer Research, **2**:28-31. 1942.

The Retention of Radioactive Phosphorus When Administered in Different Chemical Forms*†

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The efficacy of radioactive phosphorus as a therapeutic agent in some types of leukemia and allied diseases has been demonstrated by Lawrence and others (3, 2, 4). The form in which radioactive phosphorus has thus far been administered is the dibasic sodium phosphate. This was used at first orally and then intravenously (6).

That radioactive phosphorus may be administered safely in this form ($Na_2HP'O_4$) either orally or intravenously has been thoroughly established. With the development of greater efficiency in the cyclotron and particularly with the utilization of iron phosphide probes within the cyclotron rather than red phosphorus in the target field for bombardment, it has become possible to obtain phosphorus of very high specific activity, often approximating 1 mc. per mgm. of phosphorus.

Therefore, it seemed feasible to give therapeutic doses of radioactive phosphorus in varied chemical forms, since the total amount of phosphate-containing compound administered would be very small and well below the threshold of any harmful effect. Probably ionization of the phosphate compounds occurs promptly, and any therapeutic effect depends not on the particular form in which the radioactive phosphorus is administered, but rather on the radioactive phosphorus itself.

Since dibasic sodium phosphate is not readily prepared chemically from iron phosphide, since the half life of radioactive phosphorus is limited (14.3 days), and since the services of skilled chemists are difficult to obtain at the present time, it has become of practical importance to determine whether or not other more easily prepared forms containing the phosphate acid radical are satisfactory for therapeutic use.

Through the courtesy of Dr. John Irvine of the Massachusetts Institute of Technology, we obtained supplies of radioactive phosphorus in the form of magnesium ammonium phosphate and of phosphoric acid. Owing to the fact that phosphoric acid was diluted with 0.85 per cent of sodium chloride for the purpose of intravenous injection, it is probable that some formation of sodium salts may have taken place. The magnesium ammonium phosphate ($MgNH_4PO_4$) is insoluble in water or in alkaline solutions. In the very small amounts necessary for injection, it was soluble at pH 6.5 in 0.85 per cent sodium chloride and 5 per cent glucose. This solution can be autoclaved without caramelization and proved harmless, first to experimental animals, and then to human beings.

No reactions have been encountered with the intravenous administration of radioactive phosphorus in the form of phosphoric acid or of magnesium ammonium phosphate. Some pyrogenic reactions resulted from the intravenous use of lots of dibasic sodium phosphate of low specific activity that had not been completely freed from impurities. In recent months, since the preparation of magnesium ammonium phosphate entails more chemical manipulations than does that of phosphoric acid, the use of magnesium ammonium phosphate has been abandoned and phosphoric acid used entirely.

The amounts of radioactive phosphorus administered ranged from 130 to 3,850 μc. in the case of $Na_4HP'O_4$, from 1,100 to 3,900 μc. in the case of $MgNH_4P'O_4$, and from 1,000 to 4,000 μc. in the case of $H_3P'O_4$. All measurements represent microcurie equivalents and were made with a modified Geiger counter checked against the Lauritsen type electroscope as modified by Hudson and Cowing (1).

The therapeutic effect of these three compounds in the treatment of leukemia has been indistinguishable. The concentration of equivalent doses of the different compounds in separated and ashed leukemic cells has been similar within the limits of experimental

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error. The excretion rates have been essentially the same in all three, and conversely, the amount of retention in the body.

In order to demonstrate this point clearly, a set of three "scatter" graphs has been plotted, one for each of the three compounds. Fig. 1 [1] represents the percentage of retention of P^{32} administered as $Na_2HP'O_4$. Fig. 2 represents that for $MgNH_4P'O_4$. Fig. 3 represents that for $H_3P'O_4$. Each point on the graph represents a single determination of the percentage of the original dose of radioactive phosphorus administered retained in the body at that given time. The calculations have been carried out to allow for both excretion from the body and decay of radioactivity. It will be noted that the three graphs are essentially similar and might well be superimposed.

The case showing the relatively low retention in Fig. 1 has already been commented upon (5). This was a case of benzol poisoning simulating leukemia. Whether the poorer retention was chance or characteristic of the disease has not been determined, as further studies were not carried out.

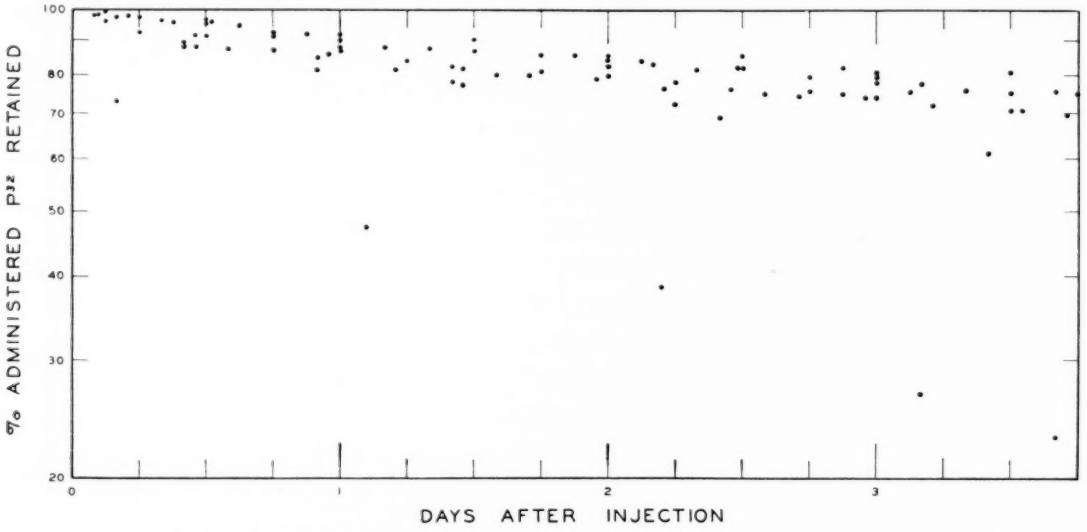

FIG. 1.—Percentage of retention of radioactive phosphorus when administered intravenously as $Na_2HP'O_4$.

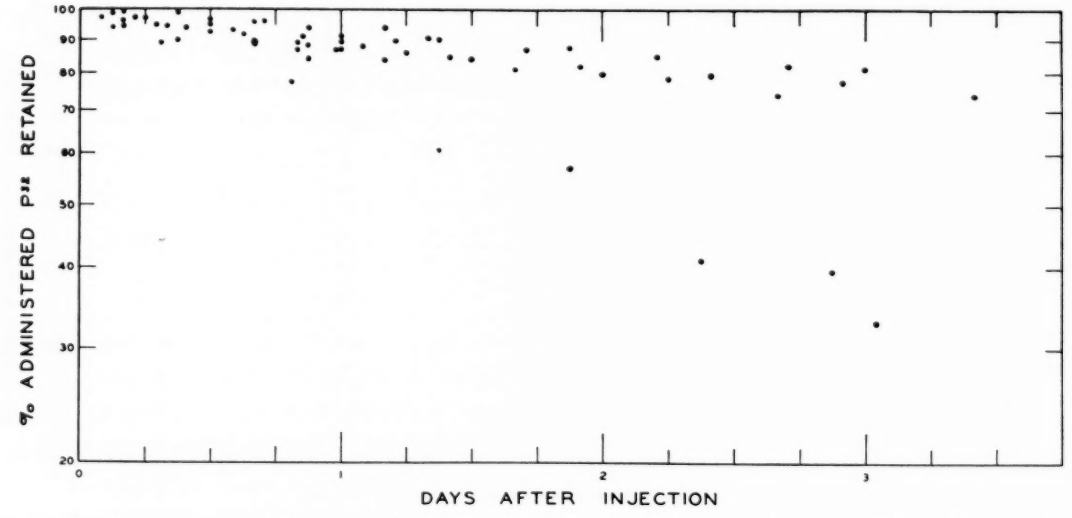

FIG. 2.—Percentage of retention of radioactive phosphorus when administered intravenously as $MgNH_4P'O_4$.

In Fig. 2 a single case shows rapid excretion. This was a dose of 3.9 mc. equivalents administered to a patient with subacute lymphatic leukemia. The patient was having severe intestinal hemorrhages. The increased loss was due to large amounts of radioactive phosphorus present in the stools, which also contained large amounts of blood. It is probable, therefore, that this does not represent loss due to true excretion, but

[1] Fig. 1 has been used in "The Retention of Radioactive Phosphorus in Leukemic Patients" (5).

loss due to hemorrhage into the gastrointestinal tract. On the administration of a number of other doses subsequently, when the clinical condition of the patient had improved and the intestinal hemorrhage was not present, the percentage of retention was indistinguishable from that of other cases.

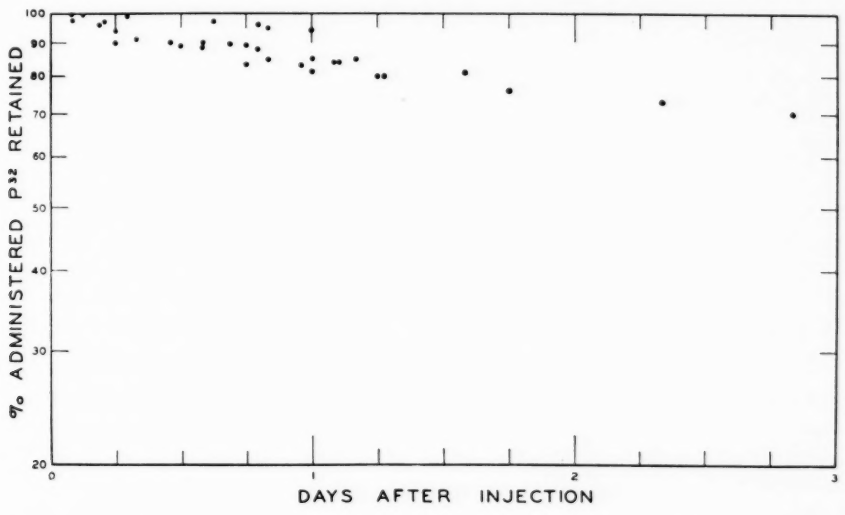

FIG. 3.—Percentage of retention of radioactive phosphorus when administered intravenously as $H_3P'O_4$.

SUMMARY

1. Small amounts of radioactive magnesium ammonium phosphate and also phosphoric acid of high specific activity may be administered safely intravenously, dissolved in 250 to 350 cc. of 0.85 per cent NaCl and 5 per cent glucose.

2. The retention of the radioactive phosphorus was practically identical regardless of which of the three compounds was used.

3. In the case of dibasic sodium phosphate and in the case of phosphoric acid one instance each of poor retention was encountered, in the former unexplained, and in the latter due to extensive intestinal hemorrhage.

4. If it is necessary to conserve time in chemical manipulations in the preparation of radioactive phosphorus for therapeutic use, H_3PO_4 or $MgNH_4PO_4$ may be substituted for $Na_2HP'O_4$ as the vehicle of administration.

REFERENCES

1. HUDSON, J. C., and COWING, R. F. Use of a Lauritsen Electroscope Under Adverse Weather Conditions. Rev. Scient. Instruments, **11**:347. 1940.
2. KENNEY, J. M. Radioactive Phosphorus as a Therapeutic Agent in Malignant Neoplastic Disease. Cancer Research, **2**:130-145. 1942.
3. LAWRENCE, J. H., SCOTT, K. G., and TUTTLE, L. W. Studies on Leukemia with the Aid of Radioactive Phosphorus. New Internat. Clin., Series 2, **3**:33-58. 1939.
4. LOW-BEER, B. V. A., LAWRENCE, J. H., and STONE, R. S. The Therapeutic Use of Artificially Produced Radioactive Substances. Radiophosphorus, Radiostrontium, Radioiodine, with Special Reference to Leukemia and Allied Diseases. Radiology, **39**:573-597. 1942.
5. WARREN, S. The Retention of Radioactive Phosphorus in Leukemic Patients. Cancer Research, **3**:872-876. 1943.
6. WARREN, S. The Treatment of Leukemia by Radio-Active Phosphorus. New England J. Med., **223**:751-754. 1940.

The Effect of Exercise on the Growth of a Mouse Tumor*

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There is considerable evidence to indicate that an inhibition of tumor growth is observed in animals maintained on a nutritionally adequate diet but limited in caloric intake (1, 5, 11, 12). This effect appears to be due entirely to the caloric restriction rather than to the lack of some specific growth factor. The result is especially significant since the animals maintained on the curtailed rations are generally in better health and outlive those allowed food *ad libitum* (5, 11). This caloric effect is not limited to laboratory animals but may pertain to human beings as well, since a review of insurance statistics concerned with the relationship of body weight to cancer incidence has shown that persons of average weight or less are not so likely to develop cancer as those who are overweight (10). It follows that the avoidance of overweight through restriction of food intake may aid in the prevention of human cancer or at least delay its onset. These observations indicate that neoplastic cells are not so likely to develop or to become established if little or no excess energy remains after the bodily requirements have been met. If this assumption is correct, it might be possible to inhibit tumor growth by subjecting animals to forced exercise and thus utilizing the excess energy. To test this possibility the influence of forced exercise on the rate of tumor growth was studied on a series of mice bearing transplantable tumors.

PROCEDURE

Young adult ABC male mice were divided into two groups, one of which was subjected to forced exercise and another kept as a control. The diet was composed of the following constituents: cerelose 71, casein 20, salts 4, corn oil 2, cod liver oil 1 and rice bran concentrate "Vitab" 2. The amount of food was limited to the amount consumed by the group that ate the least, thus assuring the same caloric intake for each group even though the food consumption varied slightly from time to time. The control groups were kept on shavings in a metal box cage while the exercised groups were placed in cylindrical wire-mesh cages 10 inches in diameter and 11 inches long each holding 25 mice. These cages were mounted in such a manner that they could be rotated by an electric motor at 2 revolutions per minute. The motor was turned on and off automatically with a special electric timing clock at any desired interval. Food and water were given when the cage was stationary. After a preliminary period of exercise of 1 or 2 weeks all mice were inoculated subcutaneously in the abdominal region with a transplantable fibrosarcoma originally obtained from the ear of a mouse which had received continued ultraviolet irradiation. The mice were weighed regularly and the size of the tumors was estimated at weekly intervals and expressed as the product of the length $\times$ depth $\times$ width in centimeters.

The experiment was conducted with two series of mice. In the first series, 100 mice were divided into two groups of 50 each; one group was subjected to exercise and the other kept as a control. Exercise was induced by rotating the cages for 16 hours continuously and was followed by a rest period for the remaining 8 hours of the day. This exercise was started 1 week before the mice were inoculated with the sarcoma. In the second series, composed of 40 mice in each group, the exercising group was rotated for 2 hours at a time with an alternate 1 hour rest period throughout the 24 hour period. The exercise was started 2 weeks before tumor inoculation and continued for the duration of the experiment.

RESULTS

It had been expected that the exercised mice would have better appetites than the control group, and although this was generally true for the first series it did not hold for the groups given the shorter rest periods. Several days after the experiment was started, it was obvious that the mice were too tired or sleepy to recover sufficiently within 1 hour to consume enough food. It was necessary therefore, to include one 4 hour period during the day to allow adequate recovery of the mice and enable them to increase their dietary consumption. Even on this schedule, however, it was

* This investigation was aided by the Jonathan Bowman Fund for Cancer Research.

usually necessary to restrict the intake of the controls to that of the exercised groups, the reverse of the expected procedure.

While the rate of rotation was not so rapid as to exhaust the mice completely, they did become very tired, and attempted to lie on the bottom of the cage until they were carried half way up to the top before it became imperative for them to return to the bottom to avoid falling. The more frequent rest periods in the second series were introduced to reduce fatigue, but as has already been said, these mice appeared too tired to be greatly interested in their food. It is probable that lack of sleep was at least as important a factor in reducing the caloric consumption as physical fatigue itself.

However, in spite of this treatment, the mice in both series remained in good health throughout the experiment, but those receiving the exercise did not gain as much as the controls on the same caloric intake. In series I the controls gained 3.8 gm. while those in the rotating cage gained only 1.9 gm. In the second series the weight gain was 3.3 gm. and 0.9 gm. respectively. This lack of a gain in weight did not appear to interfere with the general health of the exercised mice.

TABLE I: THE EFFECT OF FORCED EXERCISE ON THE GROWTH RATE OF A TRANSPLANTABLE MOUSE FIBROSARCOMA

Series	Group	No. of mice	No. of tumor takes	Weight of mice: Start of experiment, gm.	Weight of mice: Time of tumor inoculation, gm.	Weight of mice: 2 wk., gm.	Weight of mice: 3 wk., gm.	Weight of mice: 4 wk., gm.	Size of tumor, length × width × depth in cm.: 2 wk.	Size of tumor, length × width × depth in cm.: 3 wk.	Size of tumor, length × width × depth in cm.: 4 wk.
I	Controls	50	36	15.9	16.2	18.5	19.8	19.7	0.58	1.41	3.21
	Forced exercise	50	30	16.0	15.9	18.6	18.4	17.9	0.43	0.97	2.42
II	Controls	40	29	21.5	19.2	23.9	24.8	24.8	0.28	0.86	2.33
	Forced exercise	40	31	21.5	20.5	22.3	22.8	22.4	0.16	0.61	1.53

The percentage of tumor takes varied from 60 per cent to 77 per cent in both groups but was not correlated with exercise. The subsequent growth of tumors, however, was affected by exercise and is summarized in Table I. In series I the average tumor size of the controls at 4 weeks was 3.21 units (range 0.43 to 6.50 units) while that of the exercised groups was 2.42 units (range 0.20 to 6.20 units). In the second series there was likewise a slower growth rate in the rotated group at the 4th week: 2.33 units (range 0.09 to 6.81 units) for the controls as compared to the exercised group with an average of 1.53 units (range 0.03 to 3.74 units). The range of tumor size is presented to show that there was overlapping of individual tumor sizes in each group, but it should be stressed that this was true only in a relatively few cases. Such overlapping of results might be explained by differences in food intake by individual mice within a group. It would be desirable, although impractical, to conduct an experiment of this type in individual cages in order to avoid differences in caloric intake among the mice. Nevertheless, the general trend of retardation in the rate of tumor growth is quite definite in the exercised groups and is in harmony with results that demonstrate tumor inhibition in mice on calorie restricted diets.

This report is further proof of the caloric effect on tumor growth, but in addition it indicates that the influence can also be demonstrated by changing the caloric requirements of the mice while maintaining a constant dietary intake. The effect of altering the basal metabolic rate of animals, and its influence on tumor growth, have been studied by various investigators. Gilroy (2) reported that the administration of thyroxine to mice retarded cancer growth. Bischoff, Long, and Maxwell (1) did not confirm this finding but the caloric intake of the mice receiving the thyroxine was 120 per cent that of the controls. Kreyberg (4) has observed a general tendency to earlier tar tumor formation in mice given dinitrocresol or dried thyroid, while others have reported a decrease in the growth of neoplasms in animals following thyroidectomy (7, 8). Several investigators (3, 4, 6, 9) have also noted that tumors grew more slowly in hypophysectomized animals, but the general consensus of opinion indicates that the effect on tumors was directly comparable to the general body growth. Most of this work was done without attempting to control the caloric intake so direct comparisons of various experiments cannot be made, and a repetition of some of these investigations with controlled caloric feedings is definitely indicated. Nevertheless, it appears probable that all procedures that have a considerable effect on the energy requirements of the animals also influence the growth of tumors.

SUMMARY

Albino mice were placed in a motor-driven rotating cage and subjected to certain periods of forced exercise for a period preceding and following inoculation with a transplantable fibrosarcoma. The rate of growth of the tumors was then compared to a control series receiving the same daily caloric equivalent of food

but not subjected to forced exercise. The exercised mice gained less weight and the rate of tumor growth was also less than that observed in the control series.

REFERENCES

1. Bischoff, F., Long, M. L., and Maxwell, L. C. Influence of Caloric Intake upon the Growth of Sarcoma 180. Am. J. Cancer, **24**:549-553. 1935.
2. Gilroy, E. Comparison of the Effects of Arginine and Thyroxine upon Tumour Growth-Rate in the Mouse. Biochem. J., **24**:1181-1187. 1930.
3. Korteweg, R., and Thomas, F. Tumor Induction and Tumor Growth in Hypophysectomized Mice. Am. J. Cancer, **37**:36-44. 1939.
4. Kreyberg, L. Influence of Dinitrokresol on the Development of Tar Tumors in Mice. Am. J. Cancer, **36**:51-55. 1939.
5. McCay, C. M. Nutrition, Ageing and Longevity. (Nathan Lewis Hatfield Lecture.) Tr. & Stud., Coll. Physicians, Philadelphia, **10**:1-10. 1942.
6. McEuen, C. S., and Thomson, D. L. The Effect of Hypophysectomy on the Growth of the Walker Rat Tumour. Brit. J. Exper. Path., **14**:384-391. 1933.
7. McJunkin, F. A., Templeton, R. D., and Kravec, F. G. Resistance of Thyroparathyroidectomized Rats to Growth of a Spindle-Cell Sarcoma. Proc. Soc. Exper. Biol. & Med., **34**:801-804. 1936.
8. Reiss, M., and Balint, J. Thyreoidektomie und Tumorwachstum. Med. Klin., **30**:706-707. 1934.
9. Samuels, L. T., and Ball, H. A. Hypophysectomy and Tumor Growth. A Supplementary Statement. Am. J. Cancer, **23**:801-803. 1935.
10. Tannenbaum, A. Relationship of Body Weight to Cancer Incidence. Arch. Path., **30**:509-517. 1940.
11. Tannenbaum, A. The Genesis and Growth of Tumors. II. Effects of Caloric Restriction per se. Cancer Research, **2**:460-467. 1942.
12. Visscher, M. B., Ball, Z. B., Barnes, R. H., and Siversten, I. The Influence of Caloric Restriction upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48-55. 1942.

Cutaneous Carcinoma

IV. Analysis of 20 Cases in Negroes*

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Clinical and statistical studies indicate that exposure to sunlight is an important etiologic factor in the pathogenesis of cutaneous cancer in the white race, and experimental work has shown that skin cancer can be produced in animals by sunlight or ultraviolet light. The literature on cancer of the skin has been recently reviewed by several authors (1, 2, 8, 9).

In Negroes the heavily pigmented skin seems to be protected against the pathologic effects of sunlight. Clinicians have observed that skin cancer is rare in them, and this impression has been substantiated by many careful statistical studies. Skin cancer is so infrequent in the Negro race that, so far as could be determined, the literature contains no detailed report on a series of cases. An analysis of such a series should be of interest, as it may yield additional information on the etiologic factors of cancer of the skin.

This paper reports 20 cutaneous carcinomas occurring in colored male patients, with particular reference to incidence, anatomic location, pre-existing inflammatory lesions and scars, and the sex and geographic distribution of the patients. The findings on this group of colored patients are controlled by observations on white patients with cutaneous carcinoma and are compared with the statistical data reported in the literature.

The Incidence of Cutaneous Carcinoma in White and Colored Male Patients

From 1931 through 1942 the Tumor Clinic of Edward Hines, Jr. Hospital treated 10,857 white and 724 colored male patients with cancer. Cutaneous carcinoma occurred in 19.2 per cent of the white patients and in only 2.8 per cent of the colored group. The incidence of cutaneous carcinoma in Negroes is, then, only one-seventh of that observed in the white patients.

The clinical histories of the 20 cases of cutaneous carcinoma in colored men are summarized in Tables I and II as a matter of record.

Location of Lesion

In order to study the influence of sunlight in eliciting skin carcinoma, the cases were divided into two groups: (a) cancer of the exposed surfaces of the skin including face, neck, hands, and wrists; and (b) carcinoma of the covered surfaces, namely, scalp, trunk, upper and lower arms, and lower extremities.

Of the 20 cutaneous tumors in the Negro, 12, or 60 per cent, occurred on the exposed surfaces and 8, or 40 per cent, on the covered skin. In contrast, in the 2,080 white male patients with skin carcinoma, 96 per cent of the growths occurred on the exposed and only 4 per cent on the covered surfaces. This indicates that cutaneous carcinoma has a definite predilection for the exposed skin in the white but not in the colored patients.

Carcinoma of the exposed skin occurred in 18.4 per cent of the 10,857 white male patients with cancer and in only 1.7 per cent of the 724 colored men. The notable difference in percentages is statistically significant. Carcinoma of the covered skin occurred in 0.8 per cent of the white cancer patients and in 1.1 per cent of the Negroes. Evidently epithelioma of the covered skin is infrequent in both the white and colored patient, but there is no appreciable racial difference in the incidence of this type of tumor.

The findings indicate that, in comparison to the white men, the Negroes had a much lower incidence of carcinoma of the exposed skin but the same incidence of carcinoma of the covered skin.

Pre-existing Inflammatory Lesions and Scars

Of the 20 cutaneous carcinomas 5, or 25 per cent, definitely arose in a pre-existing inflammatory lesion or scar. The histories of the 5 patients are presented in detail at the end of the paper and are summarized in Tables I and II. In three additional cases the tumors probably arose at the site of pre-existing

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inflammatory lesions, but the clinical histories were not decisive. Four of the cutaneous tumors in the Negro developed in scars, and 1 followed arsenical dermatitis. The scars resulted from a variety of injuries, namely, carbuncle, ulcer associated with varicose veins, extensive burn, and a gunshot wound received in civil life. It is probable that these injuries had poor or no medical treatment. In spite of the fact that nearly all the patients in this hospital are war veterans, none of the cutaneous tumors followed a war injury.

TABLE I: A SUMMARY OF CLINICAL HISTORIES OF 20 CUTANEOUS CARCINOMAS IN NEGROES

Tumor Case No.	Site of lesion	Age	Duration	Size (cm.)	Histology	Pre-existing lesion	Initial treatment	Results
	Exposed Surfaces							
11206	Lower eyelid	46	2 yr.	1	S.	—	Excision	
6594	Nose (side)	43	3 mo.	Small	S.	—	X-ray	No recur. 4 yr. 11 mo.
12152	Nasolabial fold	47	5 yr.	9	S.	Fleshy mole, 5 yr.	X-ray. Enucleation of eye	No recur. 1 yr. 1 mo.
8223	Nose (side)	43	3 yr.	0.3	—	—	X-ray	
9022	Nose (tip)	43	—	0.5	—	—	X-ray	D. 1 mo.—Ca. lung
7608	Cheek	42	11 yr.	3.5 × 2.5	—	—	X-ray	No recur. 3 yr. 4 mo.
2977	Upper lip (skin surface)	41	Several mo.	Very small	S. I	—	Excision and radium	No recur. 7 yr.
2571	Temporal region	41	2 mo.	0.8	—	—	X-ray	No recur. 8 yr. 6 mo.
557	Neck (side)	41	7 mo.	Large	S.	—	Palliative radium and x-ray	Persistence. D. 5 mo.
8936	Neck (back)	49	25 yr.	2.5 × 2.5	B.	—	Excision	No recur. 1 yr. 8 mo.
6437	Neck (back)	67	9 yr.	12.5 × 7	B.	*	Incomplete excision and radon seeds	Persistence. D. 3 yr. 6 mo.
8911	1. Right thumb 2. Left hand †	43	5 mo.	1. 6 × 4 2. 2.5	1. S. III 2. S. II	*	1. Amputation 2. X-ray	D. 1 yr. 3 mo.
	Covered Surfaces							
7483	Scalp (parietal region)	43	9 yr.	4	S. II	Small scar following fall, 22 yr.	X-ray	Recur. D. 3 yr. 7 mo.
6118	Back of upper thorax ‡	50	3 yr.	10 × 9	B.	—	Excision	D. 4 mo. with pulmonary metastasis ?
6964	Scapular region	51	?	12 × 10	S. I	Persistent ulcer, 20 yr.	Incomplete excision	Persistence. D. 2 mo.
8256	Lower leg (posterior)	41	3 mo.	17 × 13 and metastatic nodes	S. II	*	Amputation leg. Palliative x-ray to nodes	D. 4 mo.
1879	Lower leg, inner aspect	54	7 mo.	12 × 10	S. I	*	Excision	No recur. 9 yr.
275	Popliteal space	36	13 yr.	Large	B.	—	Radium	Persistence. D. 1 yr.
11062	Thigh	50	15 ? yr.	20 × 13	S. III	*	Symptomatic	D. 41 d.
12442	Small toe (amputated in another hospital)	51	?	Large lymph nodes	S. II	?	X-ray	D. 3 mo.

Abbreviations: yr. —years
mo.—months
d. —days
S. —squamous cell carcinoma (Roman numeral after S. indicates grade of malignancy)
B. —basal cell carcinoma
Recur.—recurrence
D. —died
Ca. —carcinoma

* *See* Table II
† *See* Fig. 1
‡ *See* Fig. 2

The 5 tumors occurred a considerable period after the original injury or after the onset of the chronic inflammatory lesion. The age of the scars in which epithelioma developed varied from 8 to 31 years.

In Table III is shown the percentage of tumors

that developed in chronic inflammatory lesions or scars in colored and white patients. The statistics on the white patients were obtained in a previous study on cutaneous carcinoma in patients at Pondville Hospital (16). The table shows that 1 per cent of the white patients with carcinoma of the exposed skin, and 18 per cent of those with carcinoma of the covered skin, gave a history of a pre-existing inflammatory lesion or scar. The latter percentage is of the same order of magnitude as the 25 per cent for colored patients with cutaneous carcinoma. It is seen, then, that both cutaneous carcinoma in the Negro and carcinoma of the covered skin in the one should not assume that the combination of cutaneous carcinoma and cicatrix is fortuitous. In view of the fact that even a large scar involves only a small percentage of the surface of the skin and that many of the tumors arose definitely in the cicatrix, it would seem that the scar is an etiologic factor in cutaneous carcinoma of the Negro. On the other hand, the high frequency of scars in the control group and the rarity of cutaneous carcinoma in the colored race indicates that a cicatrix rarely gives rise to carcinoma.

The above analysis leads to the paradoxical conclusion that many cutaneous carcinomas in the Negro arise from scars, but very few scars develop carcinomas.

TABLE II: SUMMARY OF TRAUMATIC AND INFLAMMATORY LESIONS FOLLOWED BY CUTANEOUS CARCINOMA

Tumor Case No.	Age at time of trauma	Trauma or lesion	Type of healing	Time interval between lesion and tumor (years)
6437	43 and recurrence at 49	Carbuncles, back of neck	Incised with uneventful healing	8 years after 2nd lesion
8911	34	Arsenical dermatitis	Persistent	9
8256	30	Gunshot wound, right lower leg	Good healing without removal of buckshot *	11
1879	46	Ulcer with varicose veins, right lower leg	Application of iodine with inflammation and slow healing	8
11062	4	Extensive burn, back, left hip, and left thigh	No grafts	31

* *See* Fig. 3.

TABLE III: NUMBER AND PERCENTAGE OF CUTANEOUS CARCINOMAS THAT DEVELOPED IN A PRE-EXISTING SCAR OR CHRONIC INFLAMMATORY LESION IN WHITE AND COLORED PATIENTS

	Number of cutaneous carcinomas		Number developing in a pre-existing scar or chronic inflammatory lesion		Percentage	
	White	Colored	White	Colored	White	Colored
Exposed surfaces	432	12	3	2	1	17
Covered surfaces	61	8	11	3	18	38
All cases	493	20	14	5	3	25

white patient developed in a large percentage of the cases in a pre-existing scar or chronic inflammatory lesion.

A control group of 30 colored male patients in this hospital were interrogated and examined for scars. Since nearly all these patients were from Chicago, this group differs from the colored men with cutaneous carcinoma, who came from urban and rural sections of the midwest. Large cicatrices, 10 to 55 cm. in length, were found in 7, or 23 per cent, of the patients; moderate sized ones, 5 to 9 cm. in length, occurred in 10, or 33 per cent; and small ones in 9, or 30 per cent. It seems, then, that large scars are rather common in adult Negroes.

Although both the control patients and those with cutaneous carcinoma had high percentages of scars,

GEOGRAPHIC DISTRIBUTION

Patients are referred to Hines Hospital by Veterans Administration Facilities in all parts of the country, but particularly by those in the midwest. This circumstance affords an opportunity of studying the effect of geographic factors on the incidence of cutaneous carcinoma.

During 1931 through 1942, 363 colored male patients with cancer originated from the North Central states. Of these, 8, or 2.2 per cent, had cutaneous carcinoma (Table V). Of 353 patients from the Southern states, 12, or 3.4 per cent, had epithelioma. The difference in the two percentages is not statistically significant. For comparison a study of the incidence in 1941 of cutaneous cancer in the white

patients was made. Of the patients originating from the North Central and Southern states, 13.3 and 32.5 per cent respectively had cutaneous carcinoma. The difference in percentages is significant. A much higher percentage of white patients with cutaneous carcinoma came from the south than from the North Central states.

It may be concluded that geographic factors affect the incidence of epithelioma in the white but not in the colored race.

Geographic distribution.—Many investigators have observed that skin cancer in the white race occurs much more frequently in the Southern than in the Northern states (Table V). The statistics for the patients of Hines Hospital support this observation. In contrast, the incidence of cutaneous carcinoma in the Negro is not appreciably affected by geographic factors. For example, the incidence rates for white men in the United States Registration Area is 3.09 in Northern, and 5.07 in Southern states; while the

TABLE IV: INCIDENCE OF CUTANEOUS CARCINOMA IN WHITE AND COLORED MALES AND FEMALES

		Incidence rates				Ratio of incidence rates				Number of patients with cutaneous carcinoma			
		White		Colored		White : Colored		Male : Female		White		Colored	
	Years studied [2]	Male [3]	Female [4]	Male [5]	Female [6]	Male [7]	Female [8]	White [9]	Colored [10]	Male [11]	Female [12]	Male [13]	Female [14]
Patients with cutaneous carcinoma per 100,000 persons													
Philadelphia, Pa. (18)	1938	75.1	60.0	4.6	7.2	16.3	8.3	1.3	0.6	641	520	5	8
Birmingham, Ala. (17)	1938	103.9	73.2	5.7	5.6	18.2	13.1	1.4	1.0	142	103	5	5
Deaths with cutaneous carcinoma per 100,000 persons													
Metropolitan Policyholders (4)	1911–35	2.3	1.2	0.7	0.7	3.3	1.7	1.9	1.0				
U. S. Registration Area (6)	1930–32	3.35	1.92	0.96	1.03	3.5	1.9	1.7	0.9				
Patients with cutaneous carcinoma per 100 persons with cancer													
U. S. Public Health Survey													
Detroit, Mich. (11)	1937	12.3	6.2	9.2	3.0	1.3	2.1	2.0	3.1	266	212	6	5
Chicago, Ill. (3)	1937	12.5	6.9	3.7	1.3	3.4	5.6	1.8	2.9	809	464	8	5
Pittsburgh, Pa. (10)	1937	16.2	9.2	3.3	2.8	4.9	3.3	1.8	1.2	401	314	3	3
Philadelphia, Pa. (18)	1938	19.8	10.7	3.0	1.9	6.6	5.6	1.8	1.6	1,000	724	6	9
New Orleans, La. (12)	1937	29.2	20.4	4.3	2.6	6.9	7.8	1.4	1.6	369	280	8	12
Atlanta, Ga. (14)	1937	38.5	23.1	1.7	4.2	23.1	5.5	1.7	0.4	512	358	1	11
Birmingham, Ala. (17)	1938	46.1	24.9	9.3	3.5	5.0	7.1	1.9	2.7	300	211	5	6
Dallas and Fort Worth, Texas (13)	1938	47.6	23.2	6.0	2.9	7.9	8.0	2.1	2.1	836	403	3	4
Hines Hospital	1931–42	19.2		2.8			6.9			2,080		20	

REVIEW OF LITERATURE AND DISCUSSION

Incidence.—A comparison of the incidence of cutaneous carcinoma in white and colored males has been made by many investigators, who have employed three different methods for measuring incidence. Table IV summarizes the results obtained by the various authors. All the results presented in the table agree that the incidence of skin cancer in the colored person is much less than that in the white. In fact, the ratio of incidence rates of cutaneous carcinoma in white and colored individuals (see columns 7 and 8, Table IV) in the different studies is always above 1.0, and in one study it is as high as 23.1. In the present study the incidence in the white male patients was 6.9 times as great as in the colored.

rates for colored men are 1.18 and 1.03. According to the various reports in the literature, and according to the present findings, there is no significant or consistent difference in the incidence of skin cancer in the colored race for Northern and Southern states.

Sex.—In the various studies on cutaneous carcinoma in the white race, the ratio of the incidence rates for men to that for women is consistently above 1.0, and is usually approximately 1.8 (Table IV, column 9). White men, then, are more prone to develop epitheliomas than women.

In the colored race the ratio of incidence rates for men to that for women varies in different studies (Table IV, column 10). In the studies of the death rates in the United States Registration Area and

among Metropolitan Life Insurance Company policyholders the rates are low, 0.9 and 1.0 respectively. In the United States Public Health survey, which is based on smaller numbers of cases, the ratio is about 2. In spite of these inconsistencies, it is probable that there is no great difference in the incidence rates of cutaneous carcinoma in colored men and women.

Location of lesion.—It is well known that cutaneous carcinoma in white persons occurs predominantly (about 96 per cent) on the face, neck, and hands. The localization of the cutaneous lesions in colored persons has been studied by few investigators. In Quinland and Cuff's series of 11 cases (15) 5, or 45 per cent, were on the exposed skin. An analysis of Howles' data (7) shows that in 58 colored patients with cutaneous carcinoma only 41, or 71 per cent, involved the exposed skin. Similarly in this study of 20 patients the exposed surfaces of the skin were affected in 12, or 60 per cent, of the cases.

It may be concluded that there is a definite racial difference in the localization of cutaneous carcinoma.

Pre-existing lesions.—None of the published reports on cutaneous carcinoma in Negroes consider the number of tumors that develop from pre-existing chronic inflammatory lesions and scars. In a personal communication, Quinland (15) states that 3 of 11 cutaneous tumors (27 per cent) developed in pre-existing lesions (old ulcer from bubo, unhealed traumatic wound, and scar of old burn). Similarly in this study at least 5 of 20 tumors (25 per cent) arose in scars or in a chronic inflammatory lesion.

DISCUSSION

The statement is frequently made that exposure to sunlight is an important etiologic factor in cutaneous carcinoma. Strictly speaking, however, sunlight is a causal agent only in carcinoma of the exposed skin (face, neck, hands, and wrists) and is presumably not a factor in cancer of the covered skin. In view of this fact the custom of placing all cases of cancer of the skin in one group is not entirely satisfactory. It would be preferable to form two groups, namely, carcinoma of the exposed and carcinoma of the covered skin.

The division of cutaneous carcinoma into two groups led to the finding that the statistical data for carcinoma of both the exposed and covered skin in the Negro are similar to the data for carcinoma of the covered skin in the white race. Carcinoma of the exposed and covered skin in colored patients, and of the covered skin in white patients, was equally rare (1.7, 1.1, and 0.8 per cent of cancer patients respectively). Furthermore, both cutaneous carcinoma in the colored and carcinoma of the covered skin in the white men developed frequently (25 per cent and 18 per cent) in scars or chronic inflammatory lesions.

There is a factor that accounts for the similarity in the statistical data on carcinoma of the covered skin in the white patients and of the exposed and covered skin in the colored. This factor is the protection of the skin from the physiologic and pathologic effects of exposure to the sun. In the Negro the entire skin is protected by pigment, whereas in the white

TABLE V: INCIDENCE OF CUTANEOUS CARCINOMA IN WHITE AND COLORED PERSONS IN NORTHERN AND SOUTHERN SECTIONS OF THE UNITED STATES

		Incidence rates				Number of patients with cutaneous carcinoma			
		White		Colored		White		Colored	
	Year studied	Male	Female	Male	Female	Male	Female	Male	Female
Deaths with cutaneous carcinoma per 100,000 persons									
U.S. Registration Area (5)									
Northern states	1926–30	3.09	1.85	1.18	0.90				
Southern states	1926–30	5.07	3.11	1.03	1.15				
Patients with cutaneous carcinoma per 100 patients with cancer									
Northern Areas in U. S. Public Health Survey (3, 10, 11, 18)	1937–38	15.33	8.43	4.02	1.93	2,476	1,714	23	22
Southern Areas in U. S. Public Health Survey (12, 13, 14, 17)	1937–38	40.35	22.74	4.82	3.20	2,017	1,252	17	33
Hines Hospital									
North Central states	1941	13.3				131			
North Central states	1931–42			2.2				8	
Southern states	1941	32.5				131			
Southern states	1931–42			3.4				12	

race only the covered skin is shielded from sunlight by hair or clothing.

The present work affords additional evidence of the importance of sunlight in the etiology of cutaneous cancer in the white race. The findings are as clear cut as in an animal experiment. For carcinoma of the covered skin, where the influence of sunlight is excluded in the two races, the incidence is the same in white and colored patients. It seems, then, that there is no racial immunity to skin cancer. On the other hand, in carcinoma of the exposed skin the influence of sunlight is eliminated in the colored but not in the white race. The incidence of this type of cancer was found to be approximately 7 times as large in the white as in the colored patients. The findings suggest that exposure to sunlight was a major etiologic factor in six-sevenths of the carcinomas of the exposed skin in the white race.

Some of the observed differences in the statistical data for cutaneous carcinoma in the two races can be attributed to the importance of sunlight as an etiologic agent in cutaneous carcinoma of white but not of colored persons. In the white race the incidence of epithelioma is particularly great in Southern states (about 2 to 3 times as large as in Northern states) and in men (about twice as frequent as in women). In the colored race there is no definite difference in the incidence of cutaneous carcinoma in Northern and Southern states and in men and women.

The predominating etiologic factors in cutaneous carcinoma of colored individuals are scars and chronic inflammatory lesions. Although many tumors were found to arise in pre-existing lesions, it is probable that few scars give rise to carcinoma. It would seem, then, that scars and chronic inflammatory lesions are not potent carcinogenic factors.

It was surprising to observe that although nearly all the patients in this hospital are war veterans, none of the cutaneous tumors arose on the basis of a war injury. This finding may be due to the fact that war injuries usually receive adequate medical attention and are, therefore, less likely to give rise to large, irregular, distorted scars or chronic ulcers. It seems that cutaneous carcinoma develops more frequently after civilian than after war injuries.

SUMMARY AND CONCLUSIONS

This paper presents a detailed analysis of 20 colored male patients with cutaneous carcinoma and reviews the literature on this subject. From this study the following conclusions may be drawn.

Carcinoma of the exposed skin is much less frequent in colored than in white persons, but carcinoma of the covered skin has the same incidence in the two races.

In the white race cutaneous carcinoma is more prevalent in the Southern than in the Northern states, and in the colored race the incidence of cutaneous carcinoma is not affected by geographic factors.

White men have a higher incidence of cutaneous carcinoma than white women, whereas no definite sex difference in incidence was observed in the colored race.

A high percentage of cutaneous carcinomas in colored patients developed in a pre-existing scar or in a chronic inflammatory lesion. Paradoxically, a low percentage of scars give rise to an epithelioma. None of the cutaneous carcinomas in the Negro followed a war injury.

Exposure to sunlight and other climatic conditions is, it is believed, the major etiologic factor in carcinoma of the exposed skin in the white race. Scars and chronic inflammatory lesions are, apparently, important etiologic factors in carcinoma of the exposed and covered skin in the colored race, and also in carcinoma of the covered skin in the white race.

Case Histories

Case No. 6437.—The patient, a 67 year old Negro male, had a carbuncle on the back of his neck in 1914. This healed, leaving a scar. The carbuncle reappeared in 1920, was incised, and healed uneventfully. In 1928 a tumor developed in the scar. X-ray treatment at another hospital caused regression of the lesion. In September, 1936, the patient observed a hard mass about the size of a quarter at the site of the previous lesion. The mass increased in size and then ulcerated. He was admitted to this hospital on December 1, 1937. Physical examination showed an ulcerated area 12.5×7 cm. with indurated, slightly raised edges. The ulcer was deep and involved the muscles of the back and neck. There were no palpable regional lymph nodes. Several biopsies made before and during treatment resulted in the diagnosis of basal cell carcinoma.

Case No. 8911.—The patient, a 43 year old colored male, was admitted August 24, 1939, with a history of generalized arsenical dermatitis of nine years' duration. He had been treated for this condition in several different hospitals without any improvement. Five months prior to admission he developed progressively growing ulcers on both hands. On examination in this hospital he had large, warty, keratotic lesions on the buttocks, shoulders, back, and the palmar and dorsal aspects of the hands and fingers. The palmar surface of the proximal phalanx of the right thumb had an ulcer measuring 6×4 cm. There was also a raised, ulcerated lesion 2.5 cm. in diameter on the web between the thumb and index finger of the left

hand (Fig. 1), and there were large, ulcerating, metastatic nodes in both axillae. The two primary lesions were found on histological examination to be squamous cell carcinoma. The tumor on the right hand was grade 3 and on the left, grade 2. It is of interest to note that the larger lesion (6×4 cm.) had a higher grade of malignancy than the smaller lesion (2.5×2.5 cm.), although both tumors were said to be of the same duration (5 months).

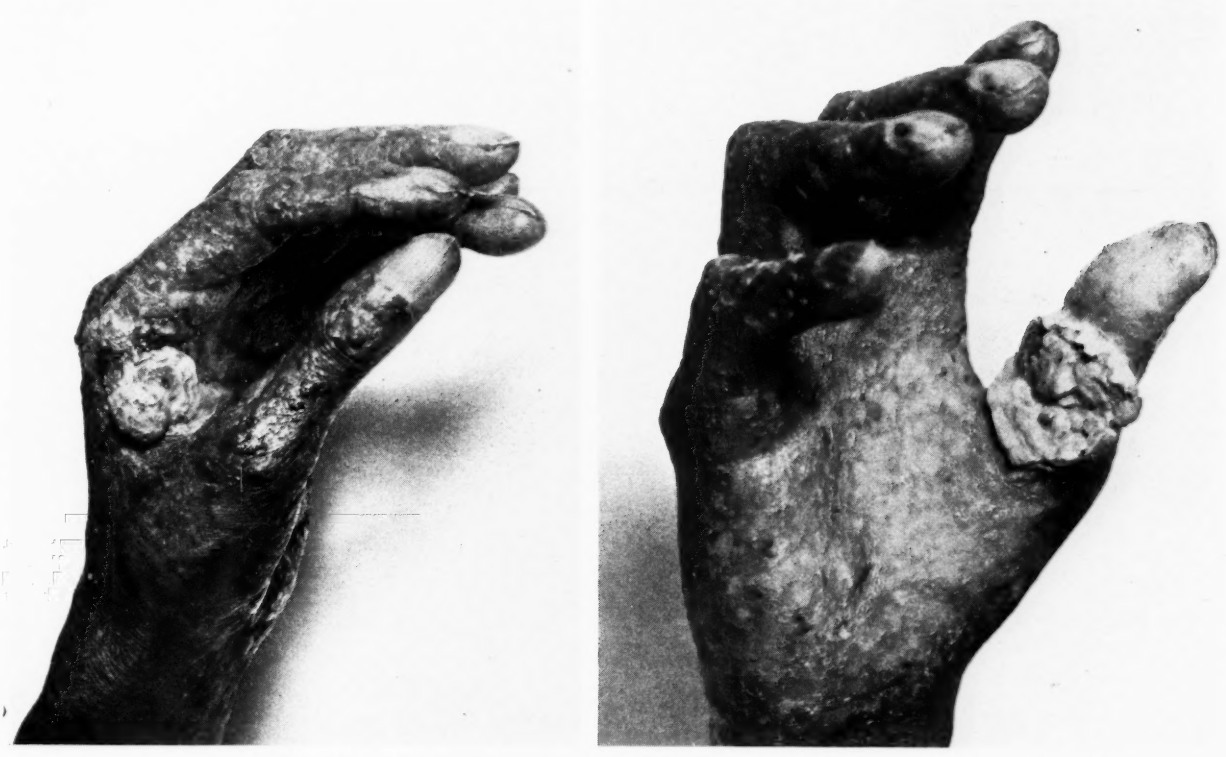

FIG. 1.—*Case 8911.* Cutaneous tumors occurring on the hands of a colored man with arsenical dermatitis.

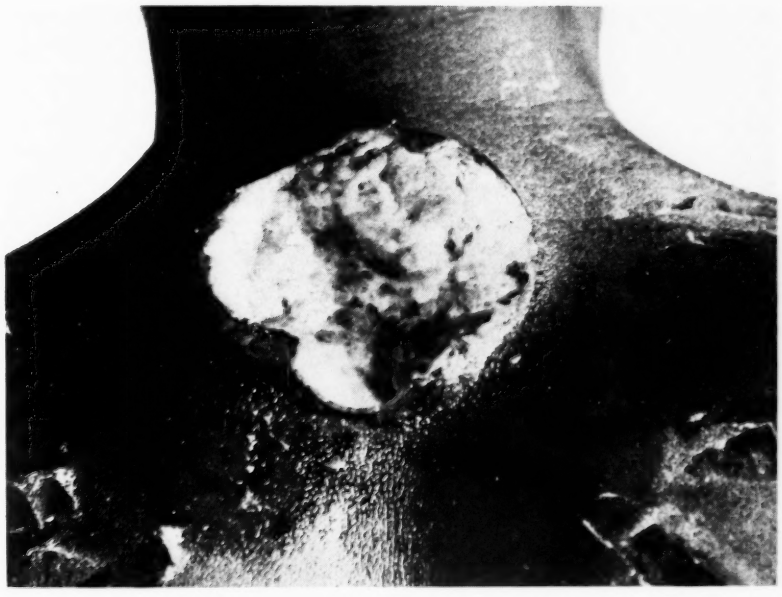

FIG. 2.—*Case 6118.* Cutaneous carcinoma on the back of a colored man.

Case No. 8256.—A 41 year old colored male patient entered the hospital March 6, 1939. He stated that in 1927 he accidentally shot himself in the right lower leg. The wound healed after a short time and left a small scar that gave him no trouble. In the first week of December, 1938, he bruised the leg on a corn stalk and there soon appeared a bluish, discolored area surrounding the old scar. Several days later the area became painful, tender, and swollen. About one

FIG. 3.—*Case 8256.* Roentgenogram of the right lower leg of a colored man with cutaneous carcinoma. The tumor is posterior to a group of buckshot that had been present for 11 years.

month after the injury an ulcer developed and grew rapidly. The following month he noticed a large mass in the right inguinal region.

On examination in this hospital the lower third of the right leg on the posterior surface was found to have a large, infiltrating, ulcerated tumor 17 × 13 cm. In the inguinal region there were firm, fixed nodes (4×5 and 10×8 cm.). On x-ray examination, there were found approximately 31 small, rounded, opaque shadows resembling buckshot anterior to the tumor in the right leg (Fig. 3). The biopsy diagnosis was carcinoma, squamous cell, grade 2.

Case No. 1879.—A 54 year old colored male was admitted to the hospital December 8, 1932. He gave a history of varicose veins since 1900. In 1924 he struck the anterior surface of the left lower leg. An abrasion developed, which was treated by the application of iodine. The area became inflamed and healed slowly but finally healed completely. In May, 1932, an ulcer developed in this area and gradually increased in size. On examination in this hospital, the inner aspect of the left lower leg just above the ankle was found to have an extensive, irregular, ulcerated lesion measuring 12×10 cm. and raised 1 cm. In addition there was considerable varicosity of the superficial veins of the left lower leg. Histological examination of the lesion showed carcinoma, squamous cell, grade 1.

Case No. 11062.—A 50 year old colored male gave a history of an extensive burn at the age of 4 years, with involvement of the left side of the back, left hip, and left thigh. The burn healed, leaving a nonsymptomatic scar. About 30 years afterward, in 1926, an ulcer developed in the scar on the thigh. The ulcer varied in size and was at times large and at other times small, but never healed completely. He was admitted to the hospital June 16, 1941.

Examination showed an extensive scar extending from the inferior angle of the left scapula, across the back and buttocks, down the posterior lateral aspect of the left thigh, and terminating in the left popliteal space. In the scar of the lateral surface of the upper thigh was an ulcer 20×13 cm. A biopsy led to the diagnosis of carcinoma, squamous cell, grade 3.

REFERENCES

1. Beerman, H., and Stokes, J. H. Photodynamic Effects in Dermatology. Am. J. M. Sc., **204**:601-624. 1942.
2. Blum, H. F. Sunlight and Cancer of the Skin. J. Nat. Cancer Inst., **1**:397-421. 1940.
3. Dorn, H. F. Incidence of Cancer in Cook County, Illinois, 1937. Pub. Health Rep., **55**:628-650. 1940.
4. Dublin, L. I., and Lotka, A. L. Twenty-Five Years of Health Progress. New York: Metropolitan Life Insurance Company, 1938.
5. Gover, M. Cancer Mortality in the United States. II. Recorded Cancer Mortality in Geographic Sections of the Death Registration States of 1920, from 1920 to 1935. Pub. Health. Bull. No. 252, 1940.
6. Gover, M. Cancer Mortality in the United States. III. Geographic Variation in Recorded Cancer Mortality for Detailed Sites, for an Average of the Years 1930-32. Pub. Health Bull. No. 257, 1940.
7. Howles, J. K. Cancer of the Skin in the Negro Race. New Orleans M. & S. J., **89**:143-145. 1936.
8. Hueper, W. C. Occupational Tumors and Allied Diseases. Baltimore: Charles C. Thomas, 1942.
9. Lewis, J. H. The Biology of the Negro. Chicago: The University of Chicago Press, 1942.
10. McDowell, A. J. Incidence of Cancer in Pittsburgh and Allegheny County, Pennsylvania, 1937. Pub. Health Rep., **55**:1419-1451. 1940.
11. McDowell, A. J. Incidence of Cancer in Detroit and Wayne County, Michigan, 1937. Pub. Health Rep., **56**: 703-739. 1941.
12. McDowell, A. J. Incidence of Cancer in New Orleans, La., 1937. Pub. Health Rep., **56**:1141-1170. 1941.
13. McDowell, A. J. Incidence of Cancer in Dallas and Fort Worth, Texas, and Surrounding Counties, 1938. Pub. Health Rep., **57**:125-139. 1942.
14. Mountin, J. W., Dorn, H. F., and Boone, B. R. Incidence of Cancer in Atlanta, Ga., and Surrounding Counties. Pub. Health Rep., **54**:1255-1273. 1939.
15. Quinland, W. S., and Cuff, J. R. Primary Carcinoma in the Negro. Anatomic Distribution of 300 Cases. Arch. Path., **30**:393-402, 1940, and personal communication.
16. Schrek, R. Cutaneous Carcinoma; Statistical Analysis with Respect to Site, Sex, and Preexisting Scars. Arch. Path., **31**:434-448. 1941.
17. Sommers, H. J. Incidence of Cancer in Birmingham and Jefferson County, Alabama, 1938. Pub. Health Rep., **57**: 377-397. 1942.
18. Sommers, H. J. Incidence of Cancer in Philadelphia, Pa., 1938. Pub. Health Rep., **57**:1843-1857. 1942.

Studies in Esterase (Butyric) Activity

III. The Effect of Foster Nursing on the Esterase Content of Blood Serum and Liver of Strains of Mice Susceptible or Insusceptible to Mammary Cancer*†

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Studies on homozygous strains of mice have resulted in a recognition of certain factors that are operative in the development of spontaneous cancer of the breast. It was shown (14) that on reciprocal breeding between strains with a high and a low tumor incidence the offspring tended to follow the tumor incidence of the mother. This predominance of the mother influence was attributed to an extrachromosomal factor. Later work (5) attributed this influence to a substance or property present in the mother's milk. It was further shown (3) that there was a correlation between the amount of milk ingested by the young animals and the frequency and the time of appearance of these tumors in later life. It was suggested that the agent in the milk responsible for this effect in fostered animals was constant in amount throughout the lactation period and that "a definite amount (threshold dose) of the milk influence was essential for the occurrence of the breast tumors in mice." The nature of the agent or agents in the milk that contributed to the development of these tumors has eluded identification so far. The present investigation was undertaken with a view to an elucidation of this problem as previous work (12) had shown that the esterase content of blood serum of a strain of mice, C57 black, with low incidence of mammary cancer was much lower than that of a strain with a very high incidence, C3H. These two strains of mice have been very carefully studied (2, 13) as regards the frequency of mammary cancer and the influence of foster nursing on it. It was found that a high percentage (66.1 to 88.2 per cent) of C3H female mice developed breast cancer in the various generations and that breeding females showed an incidence of nearly 100 per cent at an average age of 8 to 9 months. The breeding females of C57 black strain developed the same type of cancer in less than 1 per cent of the population.

Fig. 1 shows the percentage of these two strains of stock breeding female mice that died with or without cancer of the breast at successive age periods. The figure is based on data published by Andervont and by Little, Murray, and Cloudman. Reciprocal foster nursing lowered the number of spontaneous breast tumors (2) in C3H mice from 100 to 25 per cent and raised the incidence of these tumors (1) in C57 black from 1 to 15 per cent.

In the present experiment it was decided to study the effect of reciprocal foster nursing on the esterase activity in the blood serum and liver in these two strains of mice, particularly as measurements of esterase content lend themselves to accurate statistical analysis.

EXPERIMENTAL

The two strains of mice C57 black and C3H, described in a prior (5) publication, were used. A few females from each strain were selected for breeding at the commencement of this experiment. Pregnant mice derived from brother-to-sister matings were observed 3 times a day for ascertaining the birth of the litters. In most cases the litters were born at about the same time in the two strains. As the young were born, half of each litter was transferred to a foster mother of the reciprocal strain. The longest possible time during which the new born mice were left with

* The uniform reduction in the liver esterase in this series of experiments as compared with our earlier figures (7) was probably due to the change in the stock diet of the colony. This was unavoidable owing to wartime difficulties in procuring fresh whole milk. The present stock diet was modified by withdrawing the more costly item of fresh whole milk and replacing it by meat and vegetable oils.

Similar experiments have been undertaken on the C57 and A strains of mice and their findings will form the subject of a future communication.

† Because of the difficulties of international communication the authors have not read proof of this article.

** We are deeply indebted to our mathematical colleagues at this University. The results of the experiment were examined by Prof. D. D. Kosambi, Poona. We are grateful to him for the analysis of variance of our data and the conclusions based on it. Professors Maclean and Priolkar have guided our faltering progress in the application of modern statistical methods to experimental observations.

their mothers was 16 hours. The young ones were weaned from their mothers after 21 days. The males and females were separated and left in cages along with their litter mates so as to ensure identical conditions of diet and environment. They were sacrificed when they had attained the age of 6 to 8 months and the serum and liver esterase content was estimated according to the method previously described.

RESULTS

The esterase content of serum and liver of these animals is shown in Tables I and II and graphically represented in Fig. 2.

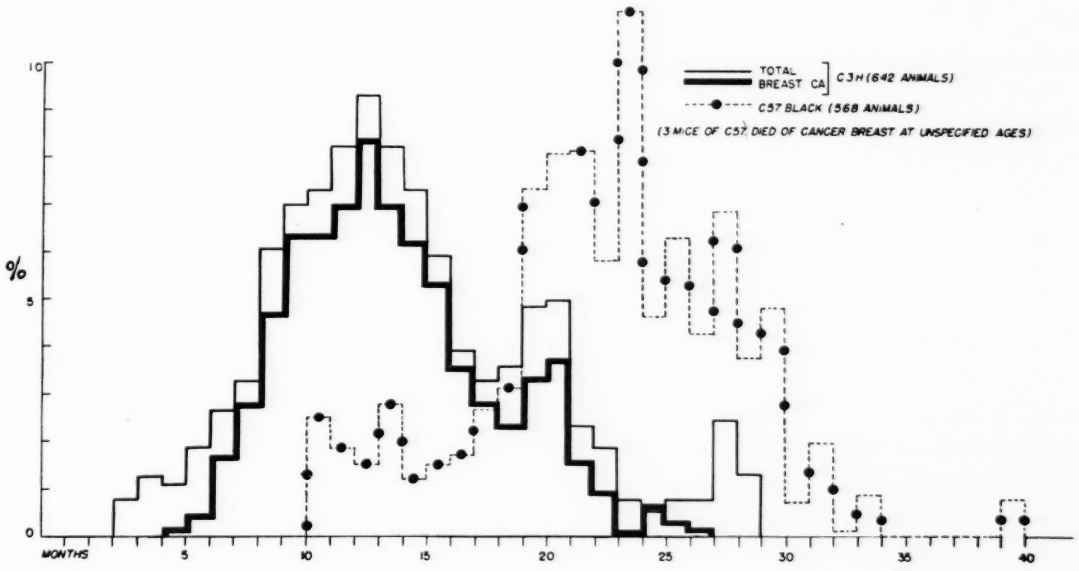

FIG. 1

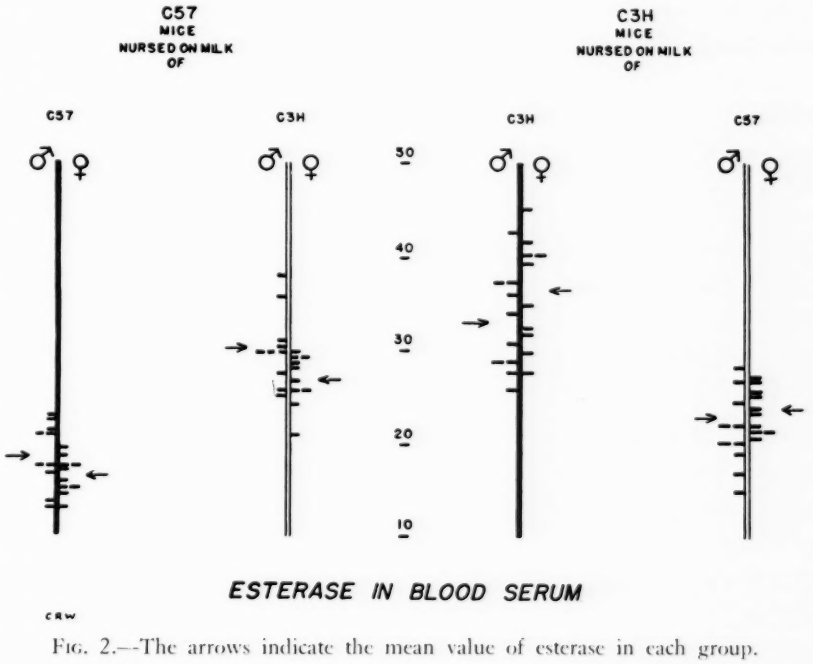

FIG. 2.—The arrows indicate the mean value of esterase in each group.

Esterase content of serum.—The mean esterase content in C57 males and females was 18.35 and 16.10 respectively. The mean esterase content in serum of C3H males was 32.85 and in the females 36.15. The

TABLE I: THE ESTERASE CONTENT OF SERUM AND LIVERS OF C57 MICE FOSTER NURSED BY C3H MOTHERS AND OF THEIR LITTER CONTROLS

Foster nursed				Litter controls			
Serial No.	Sex	Serum esterase in terms of cc. $N/100$ NaOH	Liver esterase in terms of cc. $N/10$ NaOH	Serial No.	Sex	Serum esterase in terms of cc. $N/100$ NaOH	Liver esterase in terms of cc. $N/10$ NaOH
355	M	29.5	23.5	325	M	21.0	29.8
356	"	29.5	24.4	326	"	22.5	23.6
357	"	30.5	26.2	327	"	20.5	21.2
277	"	27.5	31.7	328	"	20.5	25.1
279	"	25.0	25.7	237	"	13.0	23.3
280	"	25.5	21.1	238	"	16.5	25.2
281	"	35.5	25.6	241	"	13.5	28.8
282	"	37.5	25.0	242	"	22.0	24.0
283	"	30.0	24.1	243	"	17.0	26.5
284	"	29.5	21.5	244	"	17.0	21.6
Mean		30.0	24.88	Mean		18.35	24.9
351	F	29.0	24.2	321	F	17.0	20.0
352	"	28.0	23.4	322	"	19.0	25.0
353	"	24.0	23.3	323	"	15.0	22.8
354	"	25.5	23.8	324	"	15.5	25.0
270	"	28.5	25.0	226	"	15.0	25.6
271	"	26.5	30.5	228	"	14.5	26.0
272	"	25.5	20.6	230	"	16.8	27.9
273	"	20.5	21.2	232	"	18.2	20.8
274	"	29.5	20.0	234	"	17.0	26.7
275	"	29.0	25.5	235	"	13.0	21.8
Mean		26.6	23.75	Mean		16.1	24.16

TABLE II: THE ESTERASE CONTENT OF SERUM AND LIVERS OF C3H MICE FOSTER NURSED BY C57 MOTHERS AND OF THEIR LITTER CONTROLS

Foster nursed				Litter controls			
Serial No.	Sex	Serum esterase in terms of cc. $N/100$ NaOH	Liver esterase in terms of cc. $N/10$ NaOH	Serial No.	Sex	Serum esterase in terms of cc. $N/100$ NaOH	Liver esterase in terms of cc. $N/10$ NaOH
338	M	22.0	23.3	296	M	29.0	28.3
339	"	28.0	23.1	297	"	28.0	28.3
340	"	24.5	27.0	298	"	37.0	20.7
341	"	20.5	25.7	299	"	42.5	28.3
342	"	14.5	25.1	300	"	35.5	30.3
253	"	26.5	20.0	301	"	37.0	22.7
254	"	24.0	23.3	302	"	31.0	25.6
255	"	16.5	20.7	361	"	34.0	23.5
257	"	27.0	25.0	362	"	25.5	23.9
258	"	25.5	20.0	363	"	29.0	22.9
Mean		22.9	23.32	Mean		32.85	25.45
330	F	27.0	22.6	286	F	28.0	29.3
331	"	21.5	23.8	287	"	30.0	28.1
332	"	24.0	25.7	288	"	45.0	21.5
333	"	21.5	21.7	289	"	41.5	29.1
334	"	26.5	27.3	290	"	31.5	31.5
335	"	25.0	24.2	291	"	39.0	29.0
336	"	25.5	26.7	292	"	40.0	28.0
337	"	21.0	30.0	293	"	40.0	22.2
248	"	23.5	21.6	294	"	34.5	23.2
249	"	22.0	21.6	295	"	32.0	21.1
Mean		23.75	24.52	Mean		36.15	26.30

mean esterase content in C57 black males fostered by C3H mothers was 30.00 and in females it was 26.60. The corresponding values in C3H males and females fostered by C57 mothers were 22.9 and 23.75. On the basis of analysis of variance the standard error of each of the means of serum esterase was 1.24. It was thus seen that as a result of foster nursing by C3H (high tumor strain) females the esterase content of serum in C57 males and females was increased, whereas with foster nursing by C57 (low tumor strain) mothers the esterase content of serum in males and females of C3H was decreased.

Esterase content of liver.—The mean esterase content of livers of C57 males was 24.9 and of females 24.16. The mean esterase content of the livers of C3H males was 25.45 and of females it was 26.30. The mean esterase content of the livers of fostered C57 males by C3H mothers was 24.88 and of females 23.75. The mean esterase content of the livers of fostered C3H males and females by C57 mothers was 23.32 and 24.52. The standard error of the means for liver esterase was 0.95. The figures show that as a result of foster nursing no evident difference was observed in the liver esterase.

TABLE III

Variance due to	Degrees of freedom	Blood serum			Liver		
		Sum of squares	Mean square	F. when significant	Sum of squares	Mean square	F. when significant
Strains	1	756.45	—	48.98	4.465125	—	—
Nurse	1	2475.31	—	160.29	15.051125	—	—
Sex	1	2.81	—	—	0.036125	—	—
Strains-Nurse	1	0.05	—	(308.85)	23.653125	—	—
Strains-Sex	1	120.05	—	7.77	19.306125	—	—
Nurse-Sex	1	2.11	—	—	0.666125	—	—
Strains-Nurse-Sex	1	16.20	—	—	0.001125	—	(7974.2)
Within a group	72	1111.88	15.4428	—	645.911	8.970986	—
Total	79	4484.8675			709.089875		

The data were subjected to statistical scrutiny according to R. A. Fisher's (10) analysis of variance. The result of analysis is tabulated above.

The significance of the results is as follows: 1. The greatest effect is the increase in blood serum esterase in C57 black mice due to milk influence by fostering on nurses of C3H strain. The susceptibility for spontaneous mammary tumor of the nurse is therefore the most important factor in the esterase of blood serum. 2. The effect of strains (chromosomal) is also of great importance as the increase in serum esterase activity of C3H progeny over C57 is significant at 0.1 per cent level. 3. Sex is of no significance and the interactions, *i.e.,* the differential response with respect to one factor in the presence or absence of another, is of no statistical significance, except that the sexes respond differently in the two strains and the difference between the responses to nursing for the two strains is less than might have been expected. 4. There is virtually no effect with respect to the liver esterase content, neither variations due to the "effects" as a whole nor the correlations with blood serum activity being significant.

A numerical measure of the significant effects may be stated as follows: 1. The 40 mice nursed on C3H milk showed altogether an excess of 445 units of esterase activity in the blood over the 40 mice nursed on milk from C57 black mice. 2. The 40 mice of C3H strain developed 246 units more esterase than the 40 C57 black. 3. Finally the difference between the excess of esterase in C57 males over C57 females and the (negative) excess of esterase in C3H males over C3H females is 98 units.

DISCUSSION

As has been stated above, the foster nursing of C57 young on C3H mothers increases the incidence of spontaneous mammary carcinoma in the fostered females and a reciprocal change occurs in C3H mice fostered on C57 mothers. It has also been shown that prolonged treatment with estrogens leads to the development of mammary cancer of fostered males of C57 strains. This is probably indicated in the Strains-Sex effect in the table above. These two strains of mice show a noticeable difference in the blood esterase activity (12), which undergoes a reciprocal change on foster nursing. It has also been shown that (7) the excretion of this enzyme is much smaller in strains of mice that are susceptible to breast cancer. When all these facts are considered one is led to speculate on the probable role of an enzyme activity in the development of breast cancer in these strains of mice. The enzyme is a lipase, and one may venture to surmise that a continued higher esterase activity is an indication of altered or abnormal fat and sterol metabolism. It may further be imagined that such altered metabolism leads to the elaboration of an "endogenous factor" necessary for an atypical cell proliferation of the glandular tissue of the breast. The investigations

of Rosenheim and King (16) in sterol formulation have led to the recognition of a similarity of molecular structure between some naturally occurring sterol compounds and some very potent carcinogens. The possibility that hydrocarbons related to benzanthracene or methylcholanthrene might play a part in the causation of spontaneous cancer was suggested by the fact that many naturally occurring compounds could give rise to potent carcinogens in the laboratory. Thus methylcholanthrene was prepared independently by Wieland and Dane (18) and by Cook and Haslewood (8) by dehydrogenation of dehydronorcholene, a pentacyclic hydrocarbon obtained by Wieland and Schlichting (19) from bile acid, desoxycholic acid, by simple chemical transformation. There is as yet no proof that changes of this nature take place *in vivo*. It is necessary to consider a finding of Ghiron, later confirmed by Cook and Kennaway (9, 11), that injections of desoxycholic acid itself may produce connective tissue tumors in mice, which is suggestive of a possibility that a naturally occurring sterol molecule may pass into methylcholanthrene derivative from a disturbance of sterol metabolism under adequate enzyme activity in the body. Investigations of Bergmann (4), who showed a similar close relationship between some of the cholesterol derivatives and carcinogens, has been referred to previously.

The exact changes taking place in the body as a result of foster nursing are still not clear, but it may be assumed that it initiates a type of metabolic activity in the intestinal mucosa or the liver of the animals nursed on mothers with a high mammary cancer susceptibility and ultimately leads to tumor production in the mammary gland. Bittner (6) has suggested that chemical analysis of the milk of higher cancer stock lactating females is necessary to determine the characteristics of the mammary cancer producing influence. He believes that it may be a hormone, an enzyme, or a virus. The present investigation has shown that as a result of foster nursing serum esterase content is altered. It is reasonable, therefore, to assume that the alteration in the esterase activity of the serum is an indication of some important changes in the metabolism. It is even probable that this alteration is a manifestation of other undetected changes that might have taken place within the growing body. These suppositions cannot be substantiated at this stage but the present investigation emphasizes the necessity of a careful study of metabolism of cancer-resistant and cancer-susceptible strains and their foster nursed litters. The lack of any appreciable difference in liver esterase after reciprocal foster nursing may be assumed to mean that the livers of these strains do not differ in their capacity for manufacturing the enzyme, but in regulating its level in the blood. This probability was already discussed earlier (7).

SUMMARY

1. The effect of foster nursing on the esterase activity of serum and livers of a high mammary cancer strain (C3H) and a low mammary cancer strain (C57) was studied.

2. The reciprocal foster nursing resulted in a change in the esterase content of the serum of these mice. The esterase activity in the high mammary cancer strain diminished on foster nursing by the low mammary cancer mothers, while the esterase activity in the low mammary cancer strain increased on foster nursing by high mammary cancer mothers. These results were statistically significant.

3. No significant change was observed in the liver esterase on foster nursing.

4. A probable interpretation of these findings in relation to spontaneous mammary cancer in mice has been discussed.

REFERENCES

1. Andervont, H. B. The Influence of Foster Nursing upon the Incidence of Spontaneous Mammary Cancer in Resistant and Susceptible Mice. J. Nat. Cancer Inst., **1**: 147-153. 1940.
2. Andervont, H. B., and McEleney, W. J. Incidence of Spontaneous Tumors in a Colony of Strain C3H Mice. Pub. Health Rep., **52**:772-780. 1937.
3. Andervont, H. B., and McEleney, W. J. The Influence of Foster Nursing upon the Incidence of Spontaneous Breast Cancer in Strain C3H Mice. Pub. Health Rep., **54**:1597-1603. 1939.
4. Bergmann, W. Über vermutliche Beziehungen zwischen Cholesterin und cancerogenen Stoffen. Ztschr. f. Krebsforsch., **48**:547. 1939.
5. Bittner, J. J. Breast Cancer and Mother's Milk. Relation of Nursing to the Theory of Extra-Chromosomal Causation of Breast Cancer in Mice. A Preliminary Report. J. Hered., **28**:363-365. 1937. (Abstract.)
6. Bittner, J. J. Breast Cancer in Mice as Influenced by Nursing. J. Nat. Cancer Inst., **1**:155-168. 1940.
7. Chitre, R. G., and Khanolkar, V. R. Studies in Esterase (Butyric) Activity—II. Esterase Content of Livers of Mice and Its Excretion in Strains Susceptible and Insusceptible to Mammary Cancer. Cancer Research, **3**:88-91. 1943.
8. Cook, J. W., and Haslewood, G. A. D. Conversion of Bile Acid into Hydrocarbon Derived from 1:2 Benzanthracene. Chemistry & Industry, **38**:758-759. 1933.
9. Cook, J. W., Kennaway, E. L., and Kennaway, N. M. Production of Tumours in Mice by Deoxycholic Acid. Nature, London, **145**:627. 1940.
10. Fisher, R. A. Statistical Methods for Research Workers. Oliver and Boyd. London and Edinburgh, 1941.
11. Ghiron, V. Studies on the Carcinogenic Potency of Desoxycholic Acid in Rats. Program of the Third International Cancer Congress, September 11, 1939, p. 116.
12. Khanolkar, V. R., and Chitre, R. G. Studies in Esterase (Butyric) Activity. I. Esterase Content of Serum of Mice from Certain Cancer-Resistant and Cancer-Susceptible Strains. Cancer Research, **2**:567-570. 1942.

13. Little, C. C., Murray, W. S., and Cloudman, A. M. The Genetics of Non-Epithelial Tumor Formation in Mice. Am. J. Cancer, **36**:431-450. 1939.

14. Murray, W. S., and Little, C. C. The Genetics of Mammary Tumor Incidence in Mice. Genetics, **20**:466-496. 1935.

15. Murray, W. S., and Little, C. C. Extrachromosomal Influence in Relation to the Incidence of Mammary and Non-Mammary Tumors in Mice. Am. J. Cancer, **27**: 516-518. 1936.

16. Rosenheim, O., and King, H. Ring System of Sterols and Bile Acids. Chemistry and Industry, **51**:464-466. 1932.

17. Twombly, G. H. Breast Cancer Produced in Male Mice of the C57 (Black) Strain of Little. Proc. Soc. Exper. Biol. & Med., **44**:617-618. 1940.

18. Wieland, H., and Dane, E. The Constitution of Bile Acids. LII—The Place of Attachment of Side Chain. Ztschr. f. Physiol. Chem., **219**:240-244. 1933.

19. Wieland, H., and Schlichting, O. Ztschr. f. Physiol. Chem., **150**:273. 1925—from Cook *et al.* Am. J. Cancer, **29**:219-259. 1937.

Abstracts

Experimental Research, Animal Tumors

Ueber primäre experimentelle Sarkomatose des Bauchfells. [Experimental Peritoneal Sarcomatosis.] Askanazy, M. [Freie Vereinigung d. Schweiz. Path., Lucerne, June 15-16, 1940] *Schweiz. med. Wchnschr.*, **71**:165. 1941.

Through an error 5 rats were given a much larger amount of methylcholanthrene than was intended—1 cc. of a 1.25% solution in beef fat intraperitoneally, thrice in a period of 12 days. The only one to survive died 3½ months later with peritoneal sarcomatosis. No metastases were found.—W. H.W.

Ueber die Prinzipien der krebserregenden Wirkung des Tabaks. [The Carcinogenic Activity of Tobacco.] Roffo, A. H. [Universitätsinstitut f. exper. Med. z. Erforschung und Behandlung des Krebses, Buenos Aires] *Schweiz. med. Wchnschr.*, **71**:549-552. 1941.

Numerous experiments have proved the carcinogenicity of the benzpyrene contained in tobacco tar. As a prophylactic measure, therefore, the smoking habit must be vigorously fought.

Experiment provides an answer to the question why all smokers do not develop cancer; there is required in addition a predisposed soil. Fortunately this is not present in everyone, just as it is not present in every animal of an experiment, but it can result from many influences, and if the necessary conditions are fulfilled a smoker must inevitably die of cancer.—W. H.W.

Experimental Tumor of the Hypophysis of the White Rat. Weil, A., and Hetherington, A. W. [Inst. of Neurology, Northwestern Univ. Med. Sch., Chicago, Ill.] *J. Mt. Sinai Hosp.*, **9**:842-849. 1942.

Three cases are reported of pituitary adenoma in white rats in which carcinogenic substances had been implanted in the brain. These adenomas of the anterior lobe were combined, in one rat, with gliomas of the brain, and in a second rat, with tumor of the *pars nervosa* and of the *pars tuberalis*.—S. A. G.

Metaplasia of the Bronchial Epithelium in Rats Following Application of Benzpyrene. Thornton, T. F., Jr., and Adams, W. E. [Univ. of Chicago, Chicago, Ill.] *Cancer Research*, **4**:55-59. 1944.

A short discussion of the various theories regarding the causes of bronchogenic carcinoma is given. In addition the more recent experimental attempts to produce lung tumors are briefly reviewed. A description is given of the method employed in this experiment carried out upon rats. It consists in forming a bronchial fistula by a two stage operation for exteriorization of the lung. The fistula is then treated with 3,4–benzpyrene in olive oil 2 to 3 times a week.

A type of epithelium similar to transitional epithelium was produced a week after treatment was begun. In 2 to 3 weeks an early squamous epithelium appeared. By the end of 6 weeks metaplasia of the bronchial epithelium to a well differentiated stratified squamous type had occurred. Many animals succumbed to infection, and in many, an infection in the bronchus destroyed the epithelium.—Authors' abstract.

Der Einfluss der Kastration auf das Krebswachstum. [Influence of Castration on Tumor Growth.] Baatz, H. *Zentralbl. f. d. Gynäk.*, 1941. Abst. in *Schweiz. med. Wchnschr.*, **71**:699. 1941.

Experiments on spontaneous and transplanted tumors in animals suggest that castration will not influence favorably the course of mammary carcinoma in man. Indeed, it may even benefit the cancer by upsetting the hormonal balance.

The results of experiment accord with the clinical observation that most malignant tumors in women arise during or shortly after the menopause, and the change in hormonal relationships at that time may be regarded not only as a predisposing, but as an exciting cause.—W. H. W.

Effect of Testosterone Propionate on the Adrenals and on the Incidence of Mammary Cancer in the Paris Mouse, RIII Strain. Heiman, J. [Coll. of Physicians and Surgeons, New York, N. Y.] *Cancer Research*, **4**: 31-34. 1944.

Experiments were undertaken to note the effect of androgens on the apparent relationship existing between brown degeneration of the adrenals and mammary carcinoma in the Paris mouse. The adrenal degeneration occurred frequently in this high tumor strain but was also produced by estrinization. Mammary tumors also were produced after estrogens. In testosteronized female mice of the RIII strain the incidence of mammary carcinoma was reduced from 52.2% to 19.4%. In the same series brown degeneration of the adrenals was reduced from 80.9% in the controls to 56.5% in tumor-free treated mice. In testosteronized mice exhibiting tumors brown degeneration of the adrenals was not diminished (85.7%). The diminution of a stimulating factor in the mammary gland, and an inhibiting factor in the adrenal gland appears to represent more than a casual relationship, established perhaps, by the effects of testosterone. The lowering of tumor frequency may presumably be due to reduction of the growth activity of the mammary gland epithelium, when associated in testosteronized mice with adrenal changes.—Author's abstract.

Progesterone Treatment of Uterine and Other Abdominal Fibroids Induced in the Guinea Pig by Alpha-Estradiol. Lipschütz, A., and Maas, M. [Nat. Health Service of Rep. of Chile, Santiago, Chile] *Cancer Research*, **4**:18-32. 1944.

Abdominal fibroids produced in the female guinea pig in the course of 80 days by subcutaneously implanted pellets of α-estradiol ceased growing after a tablet of

synthetic progesterone was subsequently implanted and allowed to act simultaneously with the estrogen for 34 to 52 days.

Besides this prevention of tumorous growth there was, in the progesterone period, also a considerable regression of existing fibroids. This is inferred from the fact that the fibrous tumorous effect in animals that were exposed for 80 days to the action of estradiol alone, and subsequently for 34 to 52 days to the action of both estradiol and progesterone, was only about 58% of that found in animals sacrificed after having been exposed during 80 days to the action of estradiol alone.

Regression, *i.e.*, the difference in the average fibromatogenic effect between the estradiol group and the groups treated subsequently with progesterone, was statistically as significant as was prevention.

The therapeutic action of progesterone on existing fibroids elicited by estrogens was revealed to be much more considerable when the frequency of animals with large abdominal fibroids and especially the frequency of large fibroids per animal was compared in the different groups.

The decrease in frequency of large abdominal fibroids per animal amounted to 81% in 5 to 7½ weeks under the influence of progesterone.—Authors' summary.

Microscopic Structure of Estrogen-Induced Uterine and Other Abdominal Fibroids Treated with Progesterone. Lipschütz, A., and Schwartz, J. [Nat. Health Service of Rep. of Chile, Santiago, Chile] *Cancer Research,* **4**:24-30. 1944.

The microscopic structure of uterine and other abdominal fibroids induced in the guinea pig by the continuous action of *a*-estradiol, and subsequently subjected to the simultaneous action of progesterone, was compared with that of similar fibroids that underwent regression after withdrawal of the estrogen.

The structure of tumors that were still present 34 to 53 days after the beginning of progesterone treatment was similar to that of tumors that shrank after withdrawal of the estrogen. In both cases the most conspicuous findings were the disappearance of the spindle-shaped cells (fibroblasts) from the periphery of the tumor, and the transformation of the tumor into a uniform mass of more or less hyalinized collagenous tissue with small scattered nuclei.

The statement that under the influence of progesterone uterine or other abdominal fibroids undergo structural changes characteristic of regression and similar to those taking place after the withdrawal of estrogen, fully corroborates the conclusion drawn in the previous paper, that fibroids induced by estrogens in the course of several months begin to regress when progesterone is added subsequently and allowed to act simultaneously with the estrogenic hormone.—Authors' summary.

Accuracy and Reproducibility in the Induction of Tumors with Ultraviolet Radiation. Blum, H F. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:75-79. 1943.

It is demonstrated that high reproducibility of results is obtained in experiments on the induction of tumors with ultraviolet radiation if experimental conditions are properly controlled and the results are given suitable statistical treatment. The accuracy to be expected in comparing experimental groups is discussed. New information on the dose-response curve and the effect of animal age on tumor development is also considered.—K. R. P.

Estimation of Growth Rates of Tumors. Blum, H. F. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:21-24. 1943.

The author discusses the problem of establishing an index of tumor growth that can be used in comparative studies. From the fundamental equation for exponential growth, equations are derived for the determination of such an index.—K. R. P.

Karzinomstudien. II. Stoffwechseluntersuchungen. [Studies in Cancer. II. Metabolism.] Kallos, P. *Schweiz. Ztschr. allg. Path. u. Bakt.,* **3**:75. 1940. Abst. in *Schweiz. med. Wchnschr.,* **71**:450. 1941.

In describing his investigations on the influence of carcinogenic hydrocarbons upon the metabolism of various tissues the author says that benzpyrene exerted no effect on that of renal cortex and testis from the rat.—W. H. W.

Phosphorolysis and Synthesis of Glycogen in Animal Tissues. Shapiro, B., and Wertheimer, E. [Hebrew Univ., Jerusalem, Palestine] *Biochem. J.,* **37**:397-403. 1943.

Tumor tissue (sarcoma), though it is not generally classed as a glycogen-metabolizing tissue, displayed a high phosphorylase activity. The phosphorolytic breakdown of glycogen by this tissue is, however, very slow as compared with that of other tissues. This apparent discrepancy is due to the low phosphoglucomutase activity of tumor tissue. (The source of the sarcoma is not stated.)—E. L. K.

A Study of *d*-Amino Oxidase, Uricase, and Choline Oxidase in the Livers and in Isolated Liver Cell Nuclei of Rats Bearing Transplanted Tumors. Lan, T. H. [Univ. of Rochester Sch. of Med. and Dent., Rochester, N. Y.] *Cancer Research,* **4**:37-41. 1944.

The activities of *d*-amino acid oxidase, uricase, and choline oxidase, in livers of rats bearing transplanted hepatoma 31 and Walker carcinosarcoma 256, and in the nuclei isolated from the liver cells, were determined. Hepatoma 31 caused depletion of the apoenzyme of *d*-amino acid oxidase more than its coenzyme in the livers of rats bearing this tumor. The activities of uricase and of choline oxidase were also decreased in the livers of rats bearing transplants of hepatoma 31. The concentrations of *d*-amino acid oxidase, uricase, and choline oxidase were low in the nuclei isolated from the liver cells of rats bearing transplants of hepatoma 31. Walker carcinosarcoma 256 did not cause appreciable decrease of *d*-amino acid oxidase, uricase, or choline oxidase, in the livers of rats bearing this tumor. Choline oxidase could not be detected in the isolated liver cell nuclei of rats bearing transplants of hepatoma 31 or Walker carcinosarcoma 256, nor could it be detected in the nuclei isolated from cells of normal rat liver.—Author's abstract.

The *d*-Amino Acid Oxidase, Uricase, and Choline Oxidase in Two Transplanted Rat Tumors and in Isolated Nuclei of Tumor Cells. Lan, T. H. [Univ. of Rochester Sch. of Med. and Dent., Rochester, N. Y.] *Cancer Research,* **4**:42-44. 1944.

The activities of *d*-amino acid oxidase, uricase, and choline oxidase were determined in two tumors, hepatoma 31 and Walker carcinosarcoma 256, and the results were

compared with determinations on the same enzymes in normal rat liver. Nuclei isolated from hepatoma 31 also were studied. The amount of the apoenzyme of *d*-amino acid oxidase was low in both tumors and in nuclei isolated from hepatoma 31. Addition of the coenzyme of *d*-amino oxidase to whole tissue of hepatoma 31 raised the activity of *d*-amino oxidase from about 3% of the activity in normal rat liver to approximately 12%. Experiments with nuclei isolated from hepatoma 31 and from normal rat liver cells gave parallel results. However, addition of the coenzyme to whole tissue of the Walker carcinosarcoma 256 did not increase the activity of *d*-amino acid oxidase in this tissue, which remained almost zero.

Both hepatoma 31 and nuclei isolated from it contained very little uricase, and uricase could not be detected at all in the Walker carcinosarcoma 256. However, normal rat liver and nuclei isolated from it are fairly rich in uricase.

Choline oxidase could not be detected in transplants of hepatoma 31, in nuclei isolated from this tumor, or in Walker carcinosarcoma 256. This enzyme is present in normal rat liver tissue but absent from normal rat liver cell nuclei.—Author's abstract.

Ueber den Einfluss von Injektionen racemischen Peptids auf die Benzpyrentumoren bei Mäusen. [The Effect of Injected Racemic Peptides on Benzpyrene Tumors in Mice.] Rothlin, E., and Gehlen, W. [Pharmakol. Lab. der Sandoz A.G., Basel, Switzerland] *Schweiz. med. Wchnschr.*, **71**:327-329. 1941.

The inhibition in development of these growths described by Waldschmidt-Leitz, Mayer, and Hatschek (*Ztschr. f. physiol. Chem.*, **263**:1. 1940), could not be confirmed.—W. H. W.

Chemical Treatment of Tumors. V. Isolation of the Hemorrhage-Producing Fraction from *Serratia marcescens* (*Bacillus prodigiosus*) Culture Filtrate. Shear, M. J., Turner, F. C., Perrault, A., and Shovelton, T. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**: 81-97. 1943.

Methods are described by which the yield and purity of the hemorrhage-producing bacterial filtrate factor have been improved. A simple synthetic medium containing only 3 inorganic salts and glucose was found to support the growth of *Serratia marcescens* (*Bacillus prodigiosus*) and to be especially valuable in that it facilitated purification of the active agent by not containing the numerous complex contaminants ordinarily introduced by the usual nutrient broth. Concentration of the desired fraction from large volumes of bacterial filtrate is reported to be most easily achieved by chloroform precipitation. Repeated precipitation with ethyl alcohol followed by dialysis gave highly potent preparations.

The active fractions were rich in polysaccharides, and the best preparations were negative for protein. Results of microanalysis are given. The material is stable, its potency being retained in aqueous solution or in the dry state (lyophilized) over long periods of time. Between pH 1 and 10, none of the active agent dialyzed through cellophane; none was destroyed except in solutions of greatest acidity.

The most active preparation obtained had a potency of 130,000 m.t.u. (mouse tumor units) per cc. This is in contrast with a potency of 100 units per cc. for a commercial preparation of Coley's mixed toxins. The minimum hemorrhage-producing dose contained 0.1 μgm. of total solids.

The lethal dose of the various concentrates ranged from 100 to 1,000 times the minimum hemorrhage-producing dose.—K. R. P.

Chemical Treatment of Tumors. VI. Method Employed in Determining the Potency of Hemorrhage-Producing Bacterial Preparations. Shear, M. J., Perrault, A., and Adams, J. R., Jr. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:99-105. 1943.

This report describes the experimental development of methods for more accurate bioassay of the bacterial hemorrhage-producing factor. It was found, as expected, that the use of greater numbers of mice improved the consistency and reliability of the data. More accurate determination of the presence or absence of hemorrhage by internal examination of the tumors also contributed to the value of the assay. Testing the sterility of the tumor brei ruled out the occasional variation arising from bacterial contamination. As a further aid to consistency, unusually resistant or sensitive batches of tumors were detected by controlling each bioassay with a standard preparation of previously determined potency.—K. R. P.

Chemical Treatment of Tumors. VII. Nature of the Hemorrhage-Producing Fraction from *Serratia marcescens* (*Bacillus prodigiosus*) Culture Filtrate. Hartwell, J. L., Shear, M. J., Adams, J. R., Jr., and Perrault, A. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:107-122. 1943.

The active concentrate obtained from *Serratia marcescens* culture filtrate was purified by tryptic digestion and found to retain its activity. Elementary analysis of a number of preparations following trypsin treatment gave the following average values (%): carbon, 47.5; hydrogen, 7.1; nitrogen, 2.2; phosphorus, 1.1; "acetyl," 2.2; ash, 3.5. Methoxyl was not found. A single determination for sulfur showed less than 0.2%. Nitrogen and "acetyl" were found in quantities about half those obtained prior to digestion with trypsin.

Optimal hydrolysis of the active material yielded aldohexose, hexosamine, methylpentose, and the components of a phospholipid. Approximately two-thirds of the material consisted of sugar residues of which the greater part was aldohexose. The phospholipid was apparently in a firmly bound complex. Neither polypeptide nor protein could be demonstrated.

A number of experiments on the chemical behavior of the trypsin-digested material are reported, and the results are correlated with potency changes arising from the manipulations.

The activity of aqueous solutions was not destroyed at 37°C. From 60° to 100°C., the potency was lost at rates that increased with the temperature. Hydrolytic changes resulting from acid and alkali treatments at various temperatures were studied, and decreases in potency were detected by bioassay.—K. R. P.

Chemical Treatment of Tumors. VIII. Ultracentrifugal and Electrophoretic Analysis of the Hemorrhage-Producing Fraction from *Serratia marcescens* (*Bacillus prodigiosus*) Culture Filtrate. Kahler, H., Shear, M. J., and Hartwell, J. L. [National

Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:123-129. 1943.

Three preparations of the polysaccharide fraction from culture filtrates were analyzed. The sedimentation constant of the major component at pH 7.4 was different for each preparation. For one fraction at pH 4.1 (0.3μ), the diffusion constant was 0.89×10^{-7} cm.^{2}/sec., and the sedimentation constant at 20°C. was 70.6×10^{-13} c.g.s. units. These two values gave a molecular weight of about eight million under these conditions. The particle shape was markedly nonsymmetrical. About 90% of each preparation was found by electrophoresis and sedimentation to be made up of a single component, and the hemorrhage-producing activity resided for the most part in this material.—K. R. P.

Tierexperimentelle Versuche mit Wärmebehandlung maligner Tumoren. [Treatment of Malignant Animal Tumors with Heat.] Overgaard, K., and Okkels, H. *Nord. Medic.*, **31**:317. 1940. Abst. in *Schweiz. med. Wchnschr.*, **71**:46. 1941.

Sarcoma 180 was treated with diathermy or short waves, sometimes with x-rays as an adjuvant. Selective destruction of the tumor occurred, which was dependent upon temperature and length of treatment, but not upon frequency. A satisfactory combination was 400 to 800 r, and 42 to 43.5°C. preceding or following radiation.—W. H. W.

Specific Injurious Action of Alloxan Upon Pancreatic Islet Cells and Convoluted Tubules of the Kidney. Comparative Study in the Rabbit, Dog, and Man. Attempted Chemotherapy of Insulin-Producing Islet Cell Carcinoma in Man. Brunschwig, A., and Allen, J. G. [Univ. of Chicago, Chicago, Ill.] *Cancer Research*, **4**:45-54. 1944.

Alloxan, the ureide of mesoxalic acid, when injected intravenously, produced specific necrosis of islet cells in the pancreas and epithelium of the convoluted tubules of the kidneys in rabbits. These observations are in confirmation of the work of Dunn, Sheehan, and McLetchie.

In dogs, intravenous injection of alloxan also injured specifically the islet cells and convoluted tubules of the kidney. The islet cells in these animals, however, did not exhibit the extensive coagulation necrosis observed in rabbits.

In the dog, injury to islet cells and tubular epithelium in the kidney was manifested physiologically by diabetes mellitus and uremia. Following injection there was a brief period of hyperglycemia and then a brief period of hypoglycemia; at the end of 48 hours hyperglycemia again was present and persisted for varying intervals.

The islet cells appeared more sensitive to the effects of alloxan in that some animals exhibited hyperglycemia and relatively transient or no uremia.

In dogs, 200 to 500 mgm. of alloxan per kilo was fatal in from 1 hour to 6 days, the animal having died with definitely elevated blood glucose and blood N.P.N. After total doses of 100 to 150 mgm. per kilo the animals sometimes survived with transitory diabetes and with or without transitory impaired renal function. In one dog a sustained diabetes mellitus (over 28 days) without elevated blood N.P.N. was observed.

Four human patients with carcinomatosis, one presenting an insulin-producing islet cell carcinoma of the pancreas, received intravenous injections of alloxan. Transitory beneficial effects were observed in the patient with insulin-producing islet cell carcinoma, following injection of 600 mgm. to 1 gm. per kilo, in that attacks of hyperinsulinism were abolished for 10 to 20 days following each series of injections, whereas before the injections he had 2 to 5 severe attacks a day. In the other 3 patients also, comparably larger doses of alloxan were given than in the dogs and rabbits, with effects on the blood and sugar in only one instance. Hence it appears that the human subject is much more resistant to the action of alloxan than the dog or rabbit.—Authors' summary.

The Prothrombin Concentration in the Plasma of Normal and Leukemic Rats. Sturm, E. [Rockefeller Inst. for Med. Research, New York, N. Y.] *Cancer Research*, **4**:35-36. 1944.

Whole plasma from rats with inoculated leukemia characterized by extensive liver involvement and hemorrhagic tendency shows little difference in prothrombin time from normal rat plasma. A pronounced deviation is evident when the leukemic plasma is diluted 1:1 and 1:2 with saline and compared with normal plasma similarly diluted. The results indicate that a plasma prothrombin deficiency exists in the type of transmissible rat leukemia studied. The present observations are in accord with those of Kark and Lozner, who found that dilution of human plasma may bring out evidences of prothrombin deficiency not demonstrable on undiluted plasma.—Author's abstract.

Local Immunity in Fowl Sarcoma. Des Ligneris, M. J. A., [South African Inst. for Med. Research, Johannesburg, South Africa] *South African J. M. Sc.* **7**:184-211. 1942.

Evidence of the presence of tumor antibodies in fowl tumors is adduced from experiments with the author's sarcoma strain, which is believed to have originated in tissue cultures exposed to the action of dibenzanthracene, and the specificity of which (as compared with other fowl sarcomas) is stated to have been demonstrated by experiment.

The site of antibody formation against the tumor agent is considered, with reference to two types of tumor showing local immunity reactions. These are (1) a hard tumor showing overgrowth of fibrous tissue following tumor-cell destruction; (2) a tumor with liquefied center, forming a cyst lined by myxomatous tissue, where the liquefaction is regarded as an exaggeration of the mucinous degeneration of the stroma usually in myxosarcomas, and which is represented as an attempt at combating tumor growth.

The significance of these two types of slow-growing tumor is discussed.—A. H.

The Relation between Age, Structure, and Agent Content of Rous No. 1 Sarcomas. Carr, J. G. [Inst. of Animal Genetics, Edinburgh, Scotland] *Brit. J. Exper. Path.*, **24**:133-137. 1943.

The amount of agent extracted from Rous No. 1 sarcomas was inversely proportional to the duration of growth in the host. Tumors less than 40 days old all contained some agent, while after 40 days all tumors proved to be non-filterable. The appearance of the tumor bore no relation to the amount of agent obtained in extracts. It is pointed out that experiments designed to test for the existence of such agents are more likely to succeed if very

young grafts are used for extraction, since the increase in tumor material obtained with older grafts may not compensate for the reduction in the amount of agent that may result. It also follows that a slowly-growing tumor, though bearing a filterable agent, will be non-filterable for the greater part of its life in any host.—A. H.

Prolonged Antibody Production Following Recovery of Fowls from Rous No. 1 Sarcoma. CARR, J. G. [Inst. of Animal Genetics, Edinburgh, Scotland] *Brit. J. Exper. Path.*, **24**:138-140. 1943.

The serum of fowls tested 1 to 2 years after recovery from Rous No. 1 tumors possessed a high content of neutralizing antibodies to the Rous agent. The demonstration of active antibody in such fowls is regarded, by analogy with other virus diseases, as proof of the presence of tumor virus, although tumors are not produced.—A. H.

Mitosenschädigung durch Geschlechtshormone und das Tumorproblem. [Mitotic Disturbance Caused by Sex Hormones and Its Relation to the Cancer Problem.] VON MÖLLENDORFF, W. [Anat. Inst. d. Univ. Zürich, Switzerland] *Schweiz. med. Wchnschr.*, **71**:329-331. 1941.

Various carcinogenic hydrocarbons, in common with a number of sex hormones, elicit a characteristic abnormality in the mitosis of rabbit fibroblasts *in vitro;* some of the chromosomes are excluded from the equatorial plate and take no further part in cell division. The course of mitosis is not disturbed, however, and the division rate is not materially reduced.

This irregularity, which the author regards as the most significant primary disturbance in neoplasia, he refers to the action of some substance related to the steroids.—W. H. W.

Method for the Quantitative Morphologic Analysis of Tissues. CHALKLEY, H. W. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:47-53. 1943.

A method is described for ascertaining nucleocytoplasmic ratios without resort to tedious drawing and size measurement technics. It has as its basis the probability that the ratio between the sums of large numbers of hits and misses of random points upon nuclei and cytoplasms will correspond to the ratio between the sizes of these cell components. The procedure is not limited to this problem alone but can be used to ascertain the proportions of the fractions of total volume occupied by any morphologic constituent of the tissue or organ studied. Tests of the method that show its degree of accuracy when applied to the analysis of fixed and stained preparations are presented.—K. R. P.

A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. VI. Epidermoid Carcinomas and Associated Tumors in Mice of the F_4-F_7 Generations of the NH Descent. WILLIAMS, W. L., and STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research*, **4**:11-17. 1944.

Tumors developed in approximately 80% of the NH mice that had received a single subcutaneous injection at 60 days of age of 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil. In the 694 tumor-bearing animals presented here the order of frequency of tumor types was: fibrosarcoma, epidermoid carcinoma, mammary carcinoma, and bronchiogenic carcinoma. The epidermoid growths were all squamous cell carcinomas. These were present in 34.7% of the tumor-bearing animals and tended to occur earlier than the other types. In the animals that showed only skin tumors the average latent period was 115 days, and 84.7% of the neoplasms were evident upon gross examination made prior to 150 days after injection with carcinogen. For the total group of fibrosarcomas (animals without skin or mammary tumors) the average latent period was 180 days, and 50.5% of the tumors appeared within 150 days. The frequency of epidermoid carcinomas was greater in the NHO mice than has been reported for other strains of mice and for other rodents treated in a similar manner with methylcholanthrene.—Authors' summary.

On the Genetic Character of Neoplastic Cells As Determined in Transplantation Experiments with Notes on the Somatic Mutation Theory. [Cornell Univ. Med. Coll., New York, N. Y.] FURTH, J., BOON, M. C., and KALISS, N., *Cancer Research*, **4**:1-10. 1944.

A genetic analysis of cells from spontaneous and induced neoplasms was made by studying their transplantation pattern in inbred stocks of mice and hybrids of known genetic composition. This was compared with the behavior of grafts of normal spleen. Tumors induced in the Ak or Rf strains were found to grow well in hybrids of these stocks. They may grow in both inbred lines or only in the line of origin. Tumors induced in the hybrids grew in hybrids and in both, or one, or neither of the parental lines. Tumors arising spontaneously in these lines were transplantable to the F_1 hybrids and to the line of origin, but not to the unrelated line. The spontaneous tumors arising in hybrids that were studied grew in hybrids and grew well in one of the parental lines, but not at all in the other line, or grew poorly in both. Spontaneous and induced leukemias arising in the Ak stock grew well in the line of origin and fairly well in the unrelated C3H inbred stock. Leukemias arising in the F_1 hybrids between the Ak and C3H stocks grew in F_1 hybrids and in both parental lines. The same relationship exists between Ak and C3H mice with regard to transplantability of neoplasms other than leukemia.

The conclusion is reached that the transplantation pattern of leukemias and tumors arising in inbred stocks may differ from that of normal splenic tissue, which uniformly follows a single scheme. Induced leukemias and tumors may differ greatly among themselves, but there is a transplantation pattern characteristic of each neoplasm, which is retained through numerous passages. These observations suggest that the immediate change that makes some cells cancerous may be a somatic mutation.—Authors' abstract.

The Role of Genetics in Cancer Research. BLANK, F. [Columbus, Ohio] *Ohio State M. J.*, **37**:947-951. 1941.

The author points out that even the discovery of a direct external causative agent of cancer would not do away with the need to explain individual susceptibility. He quotes from Waaler's statistical survey of the familial incidence of cancer, concluding that different types and sites of tumor growth behave in a different manner genetically. Studies of identical twins show that there is a definite

hereditary localization factor for certain types of tumors. Reference is made to investigations with inbred mouse strains showing that undoubtedly different genetic behavior in susceptibility to tumors of a particular type exists. Most workers agree that there is an inheritable specificity for tumor type and tumor site. However the factor of heredity is not the sole cause of cancer growth, and other influences play a role. Other aspects of cancer research mentioned briefly are the role of a virus, carcinogenic substances, and the influence of the endocrine glands. Recognition of the effects of heredity in the causation of cancer may lead to the development of some means of prophylaxis.—E. E. S.

The William Henry Welch Lecture. I. The Conditions Determining Cancer. Rous, P. [Rockefeller Inst. for Med. Research, New York, N. Y.] *J. Mt. Sinai Hosp.*, **8**:184-185. 1941.

The William Henry Welch Lecture. II. The Known Causes of Cancer. Rous, P. [Rockefeller Inst. for Med. Research, New York, N. Y.] *J. Mt. Sinai Hosp.*, **8**:186-187. 1941.

Abstracts of general discussions.—A. Cnl.

Clinical and Pathological Reports

Heredity

Familial Polyposis of the Colon. Falk, V. S. [Carle Memorial Hosp., Urbana, Ill.] *Arch. Surg.*, **45**:123-128. 1942.

In a family of 7 children 6 were found to have polyposis. In 2, malignant change developed in the polyps. The father of the children died of carcinoma of the rectum at the age of 48, and the paternal grandfather succumbed to "cholera morbus." The author recommends early and radical operation as the treatment of choice.—G. H. H.

Therapy—General

Surgical Relief of Intractable Pain in the Upper Extremity Due to Malignant Disease. Weinberger, L. M. [Michael Reese Hosp., Chicago, Ill.] *Ohio State M. J.*, **37**:765-767. 1941.

The author emphasizes the danger in relying on morphine for relief of severe pain associated with carcinoma of the mammary gland since many patients may survive for a long period. The pain is often due to invasion of the brachial plexus. The procedure of choice was found to be intraspinal division of dorsal roots. Other procedures are discussed, and objections to their use offered. An illustrative case is presented.—E. E. S.

Radiation—Diagnosis and Therapy

Sarcoma of the Neck Following Roentgen Therapy in Graves' Disease. Arnheim, E. E. [New York, N. Y.] *J. Mt. Sinai Hosp.*, **9**:84-86. 1942.

The first reported case of sarcoma of the neck following roentgen therapy of Graves' disease is described. Degenerative radiation changes (pigmentation and telangiectasis) in the skin of the neck occurred 2 years after the treatment. Eighteen years later a fibrosarcoma developed at the site of these skin changes. Five months after excision of the tumor a recurrence was noted. A radical neck dissection resulted in freedom from recurrence for a period of 23 months.—S. A. G.

The Effect of Roentgen Therapy in Primary Cancer of the Breast. Harris, W. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **8**:606-611. 1942.

Seventy-five patients with proved cancer of the breast received intensive preoperative roentgen therapy. Pathologic studies after subsequent mastectomy revealed no evidence of the primary tumor in 13% of the cases. The fact that 63% of the axillary nodes were found to be involved indicates that no cancerocidal action of the radiation occurred in these deposits. In view of these findings the author believes that radical mastectomy should be recommended as the treatment of choice in operable cancer of the breast without the delay and questionable benefit of preoperative roentgen therapy.—S. A. G.

Irradiation in Treatment of Malignant Tumors. Holmes, G. W. [Massachusetts Gen. Hosp., Boston, Mass.] *Northwest Med.*, **41**:264-269. 1942.

Treatment by radiation can be effected by radium, radium emanation, and low, high, and super voltage roentgen rays. The selection of the method to be used depends on the convenience of application and upon the depth and size of the tumor. Irradiation causes degeneration of tumor cells and premature aging, *i.e.*, fibrosis, of the tumor bed. There is no evidence that tumor growth is stimulated by such treatment. Tumors originating from the white blood cells, blood forming organs, gonads, and tissues of embryonic type are the most sensitive. Tumors arising from nerve tissue, bone, or muscle are characteristically resistant. A few general observations concerning technic are made. The author believes preoperative irradiation has some beneficial effect. Postoperative irradiation should be attempted only when it is known that the tumor is of a high grade of malignancy or when surgical removal is incomplete. The majority of patients referred for radiation therapy are incurable. Treatment in these cases is purely palliative and systemic reactions should be avoided. The hazards of treatment are injury to normal tissues and systemic reactions such as roentgen sickness and injury to blood-forming organs, but the latter is regarded as of minor importance. Any source of infection should be removed before treatment is undertaken.—E. E. S.

Malignancy Subsequent to Irradiation of the Uterus for Benign Conditions. Scheffey, L. C. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Am. J. Obst. & Gynec.*, **44**:925-951. 1942.

The author reviews 20 cases in which cancer of the cervix or corpus uteri followed irradiation therapy and concludes that the previous irradiation of the benign condition was probably not the cause of the malignant change.—A. K.

Radiation Cancer. Silverstone, S. M. [Mt. Sinai, Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **9**:74-83. 1942.

The various types of radiation cancer, both human and experimental, have certain fundamental features in common. The exact amount or dose of radiation necessary for the production of radiation cancer is not known, nor is it

so important as the fact that the radiation must be administered over a long period of time, either continuously or intermittently.

If radiation is given in doses sufficient to destroy the tissue irreparably, a condition known as radionecrosis is produced. Radionecrotic tissue, being practically devoid of any viable elements, seldom undergoes malignant change, but cancer may occur in the viable tissue at the edge of a radionecrotic area. However, if the dose of radiation is such as to produce incomplete destruction of various tissue components, then a complex balance of necrosis and repair is set up that may eventually lead to malignant neoplasm formation. The injury so produced in the irradiated tissue is of such character that degenerative and regenerative processes occur concomitantly. Both processes are progressive and continue indefinitely even though the injurious agent may be withdrawn, thus accounting for the extremely variable and unpredictable length of time between the administration of radiation and the appearance of the neoplasm. The histologic character of the tumor is dependent, not upon the nature of the injurious agent, but upon the type of tissue affected by radiation. The neoplasm is limited in origin to the irradiated tissue, but once established, it behaves like any other malignant growth of similar histology.—S. A. G.

Uses and Abuses of Radiation Therapy in Obstetrics and Gynecology. Traut, H. F. [New York Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.* **44**:638-647. 1942.

The use of x-ray therapy is seldom advisable in gynecologic cases during the first 2 decades of life except in the rare instances of malignant neoplastic disease. The treatment of carcinoma of the cervix constitutes the greatest use that can be made of irradiation during the reproductive period of life. It is the best mode of therapy in all stages of this disease. It is felt, however, that by semi-annual examination the disease could be detected in its incipiency, and relatively simple therapeutic methods be used. Carcinoma of the vulva should never be treated by any form of radiation therapy because of possible skin reaction. In the advanced stage of malignancy regression of the growth and relief from pain may be achieved by radiation therapy. X-ray treatment of desperately ill or very aged patients is not recommended. A knowledge of the life cycle of the tumors and their sensitiveness to radiation therapy are prerequisite to the treatment of these lesions.—A. K.

Skin and Subcutaneous Tissue

Sebaceous Gland Carcinoma. Beach, A., and Severane, A. O. [San Antonio, Tex.] *Ann. Surg.*, **115**:258-266. 1942.

Forty previously reported case studies of sebaceous gland carcinoma are reviewed and another case is added. The treatment proposed is: complete surgical excision of the primary tumor, regional node dissection, and roentgenotherapy when indicated.—M. R. D.

Cutaneous Metastases of Carcinoma. Grinevich, V. [Rostov-on-the-Don, U.S.S.R.] *Urol. & Cutan. Rev.*, **45**:514-517. 1941.

The author describes a patient with 46 adenocarcinomatous skin metastases that were thought to be from an ovarian tumor because a pelvic mass was present. Cutaneous metastases are so rare that the simple explanation of seeding is not adequate. If seeding were involved, with the present frequency of cancer, cutaneous metastases would be common. The idea is discussed that a histological affinity is necessary between the tissues at the site of primary and secondary lesions, but it is pointed out that primary cutaneous cancers almost never metastasize to skin. Cutaneous metastases usually grow rapidly. A plea is made for histological examination of all removed skin lumps.—V. F. M.

Psoriasis with Tumor-Like Formations. Observations over a Twenty Year Period. Levin, O. L., and Behrman, H. T. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **7**:449-458. 1941.

The case reported is unique in that it presents in association with psoriasis the hitherto undescribed feature of tumor formation. This type of psoriasis bears a clinical resemblance to other dermatoses and in many ways suggests sarcoma, especially Kaposi's hemorrhagic sarcoma, but biopsies show that it is not the same.—A. Cnl.

An Unusual Epithelioma of the Leg. Levin, O. L., and Behrman, H. T. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **9**:87-95. 1942.

An unusual case of malignant tumors and large ulcers of the leg is reported. The histopathologic studies made prior to the institution of therapy revealed evidence of immature squamous cell carcinoma and amelanotic melanoma. Following intensive roentgen-ray therapy there was temporary retardation of growth and partial involution of the tumors. Amputation became imperative, however, because of extension of the ulcers and the occurrence of intense pain. Histopathologic study of the tissue after amputation revealed no further evidence of malignant growth, probably as a result of the intensive radiotherapy. Examination 10 months later showed no evidence of regional adenopathy or of metastases in other regions of the body.

The differential diagnosis and therapy of these lesions are discussed.—S. A. G.

Nervous System

Tumor of the Iter and Fourth Ventricle Associated with a Meningo-Myelocele and Absence of the Cerebellum in an Infant of Five Weeks. Alpers, B. J. [Jefferson Med. Coll., and Univ. of Pennsylvania, Philadelphia, Pa.] *J. Mt. Sinai Hosp.*, **9**:296-298. 1942.

The occurrence of a large tumor in the iter and fourth ventricle of a 5 week old infant with a meningomyelocele and cerebellar aplasia is reported. The case described appears to lend support to the theory of the congenital origin of some of the infiltrating tumors of the brain.—S. A. G.

Ganglion Cell Tumor (Ganglioglioma) in the Third Ventricle. Operative Removal With Clinical Recovery. Anderson, F. M., and Adelstein, L. J. [Los Angeles County Hosp., and Univ. of Southern California Sch. of Med., Los Angeles, Calif.] *Arch. Surg.*, **45**:129-139. 1942.

A brief review of the literature and report of a case. The patient showed no signs of recurrence 18 months after operative removal of the tumor.—G. H. H.

Multiple Calcified Intraventricular Meningiomas. Case Report. COHEN, I. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **7**:329-333. 1941.

Single meningiomas lying entirely within one of the ventricular cavities constitute a very small percentage of the total number of intracranial meningiomas. About 40 intraventricular meningiomas have been reported so far; Cushing encountered but 2 in his 295 cases of intracranial meningiomas. Some of these intraventricular tumors are densely calcified, but the majority of them are not. Multiple intraventricular meningiomas are exceedingly rare. A patient with multiple calcified intraventricular meningiomas, who had been operated upon, is reported by the author. The calcification was seen on the flat x-ray plate of the skull and, after delineation by injection of air, in both lateral ventricles and in the third ventricle.

There is nothing characteristic about the symptomatology or physical signs in this type of tumor. Localizing signs are absent, and accurate localization will depend upon air ventriculography. The fact that the sole blood supply comes from the choroid plexus makes the lesion particularly favorable for extirpation.—A. Cnl.

Atypical Acoustic Neuromas. FRIEDMAN, E. D. [New York Univ. Sch. of Med., and Bellevue Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **9**:435-445. 1942.

Seven cases of atypical acoustic neuroma are described. The importance of an accurate history giving the chronologic march of events is emphasized, and reasons for errors in diagnosis are suggested.—S. A. G.

FEMALE GENITAL TRACT

Adenoacanthoma with Ovarian Metastases. BEECHAM, C. T., and FRIDAY, R. H. [Temple Univ. Hosp., Philadelphia, Pa.] *Am. J. Obst. & Gynec.* **44**:512-515. 1942.

This is a report of an adenocarcinoma of the uterine fundus, of a low grade of malignancy, which metastasized to the ovary where it produced keratin. This case is in keeping with the Novak statement of that squamous cell metaplasia occurs "in adenocarcinoma of the lesser degrees of malignancy."—A. K.

Chorionepithelioma. An Unusual Case in Which Cerebral Metastasis Occurred Four Years After Hysterectomy. DOCKERTY, M. B., and CRAIG, W. McK. [Mayo Clinic, Rochester, Minn.] *Am. J. Obst. & Gynec.*, **44**: 497-501. 1942.

A case report.—A. K.

Postmenopausal Endometriosis. A Case Report and Review of the Literature. FRANK, I. L., and GEIST, S. H. [Mt. Sinai Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **44**:652-657. 1942.

Theories of the origin of endometriosis are reviewed, and the pathologic role of estrogen stimulation is discussed. In a series of 203 cases of adenomyosis, 23 were in women past the climacteric. An additional case is reported. In the women past the menopause there were no symptoms attributable to the adenomyoma itself. There is no evidence to indicate that these tumors can originate *de novo* after the climacteric, or even persist in active, symptom-producing growth. Although uterine endometriosis is not infrequent in women past the menopause, such lesions are to be regarded as in a state of regression.—A. K.

Cancer of the Vulva. MCDONOUGH, J. J. [Youngstown, Ohio] *Ohio State M. J.*, **38**:1022-1024. 1942.

This is a statistical analysis of the cases of carcinoma of the vulva, treated at Woman's Hospital, Detroit, between 1932 and 1941. This tumor constitutes about 5% of all carcinomas occurring in organs of the female pelvis. When the patients were seen, 71% already had metastases in the regional lymph nodes. Leukoplakic vulvitis preceded development of the tumor in more than half the patients. Vulvectomy is, therefore, recommended for this precancerous condition. Pain and burning on urination, vulvar sore, bleeding, and vaginal discharge were the most common symptoms. The tumors either projected in papillary form or were seen as indurated ulcers. Secondary carcinoma in this location was very rare. Radical vulvectomy is considered the therapeutic procedure of choice.—E. E. S.

Two Unusual Cases of Chorioepithelioma. I. A Very Young Myometrial Chorioepithelioma Four Months After a Hydatid Mole in a Primigravida of Twenty-three Years. 2. An Advanced Chorioepithelioma Localized in the Cervix in a Gravida XVII of Forty-four Years With no Antecedent Hydatid Mole. RUBIN, I. C. [Mt. Sinai Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **41**:1063-1068. 1941.

A report of two cases.—A. K.

Primary Epidermoid Carcinoma of the Vulva Complicating Pregnancy. SHANNON, W. F., and MARTING, E. [Univ. of Cincinnati Coll. of Med., and Cincinnati Gen. Hosp., Cincinnati, Ohio] *Am. J. Obst. & Gynec.*, **41**:117-121. 1941.

A case report of a primary epidermoid carcinoma of the labia majora complicating a 6½ months' pregnancy in a woman 26 years of age. Treatment was simple excision of the tumor, cesarean section, bilateral radical groin dissection, and postoperative x-ray therapy. Five similar cases were collected from the literature.—A. K.

The Etiologic and Pathologic Factors in a Series of 1,741 Fibromyomas of the Uterus. TORPIN, R., PUND, E., and PEEPLES, W. J. [Univ. of Georgia Sch. of Med., Augusta, Ga.] *Am. J. Obst. & Gynec.*, **44**:569-574. 1942.

The incidence of fibromyomas in negro women near Augusta, Ga., was 3⅓ times that in white women. In the Negro women, the tumors were larger, and chronic salpingitis in conjunction with fibromyomas was encountered more often. Tumor necrosis was twice as frequent as in white women. Fibromyomas were found to be relatively free from malignant complications, but sarcoma was more frequent in the Negro, carcinoma more common in white women. The complicating ovarian changes found relatively frequently were follicular and luteal cysts.—A. K.

Cancer of the Cervix Following Supravaginal Hysterectomy. WARD, G. G. [New York, N. Y.] *Am. J. Obst. & Gynec.*, **41**:660-663. 1941.

It was found by the author that carcinoma of the stump is neither more serious nor more difficult to treat than cancer of the cervix with the fundus present except that there may be an increased frequency of vesicovaginal fistula. If castration is performed in conjunction with subtotal hysterectomy, diminished circulation might act as a retarding factor in the development of cancer of the stump. The author considers, however, that total hysterectomy is the preferable procedure in carcinoma of the corpus,

but that this is true only for the experienced surgeon. For the less experienced operator the subtotal procedure in conjunction with detailed examination of the cervix is to be recommended. In all subtotal operations careful subsequent observation of the patient for an extended period of time is essential for safety.—A. K.

Simplified Suction for Obtaining Endometrial Biopsies. WINKELSTEIN, L. B. [Stuyvesant Polyclinic, New York, N. Y.] *Am. J. Obst. & Gynec.*, **42**:163-164. 1941.

Description of an instrument combining curettement and suction for endometrial biopsy.—A. K.

URINARY SYSTEM—MALE AND FEMALE

Occult Carcinoma of the Kidney With Metastases Simulating a Primary Carcinoma of the Nasopharynx. KOLETSKY, S. [Western Reserve Univ. and University Hosp., Cleveland, Ohio] *Ohio State M. J.*, **37**:1180. 1941.

The tumor appeared in the nasopharynx of a 23 year old boy and rapidly spread to mouth, sphenoid, ethmoid, and temporal bones. Necropsy revealed a carcinoma in the kidney, extending into the renal vein. The tumor nodules in the lymph nodes, liver, and vertebrae, as well as those noted clinically in the skull, were regarded as secondary to the kidney lesion.—E. E. S.

A Carcinoma of the Kidney Following A Fracture of the Subjacent 11th Rib. MOORE, T. [Manchester, England] *Urol. & Cutan. Rev.*, **47**:2-3. 1943.

A report of the removal of a papillary carcinoma of the kidney 20 months after fracture of the overlying rib.—V. F. M.

INTRATHORACIC TUMORS—LUNGS—PLEURA

Carcinoma of the Lung. ADAMS, R. [Lahey Clinic, Boston, Mass.] *Surg. Clin. North America*, **22**:703-707. 1942.

Control or arrest of carcinoma of the lung in its advanced stages has proved almost impossible. This type of malignant growth has shown notable resistance to x-radiation, and the benefit obtained has been so limited that x-ray treatment has been discarded as a curative procedure. X-rays and radium have the same physical effects, but the technical ease of application makes x-ray therapy to the lung preferable. The only possible hope for the patient with carcinoma of the lung lies in the chance that the diagnosis has been made early enough to allow complete surgical removal of the tumor before metastases have occurred. The symptomatology, diagnosis, and surgical treatment of the disease are discussed.—J. L. M.

Localized Tuberculous Pleural Effusion Simulating Pulmonary Neoplasm. AUFSES, A. H. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **7**:626-628. 1941.

A case report.—A. Cnl.

Clinical Aspects of Malignancy of the Lung. Case Reports. BERGHAUSEN, O. [Cincinnati, Ohio] *Ohio State M. J.*, **37**:757-760. 1941.

In one patient no symptoms or signs referable to the changes in the lung were evident. The carcinoma was recognized only on histologic examination of the wall of a lung abscess. In the second patient reported, clinical symptoms were lacking despite the presence of a large bronchogenic tumor giving rise to atelectasis and multiple abscesses. Two additional patients with classic history, and roentgen ray findings, in whom the diagnosis of bronchogenic carcinoma was confirmed at necropsy, are briefly described. A fifth patient presumably had metastases in the lungs from a sarcoma of the knee, and the sixth was thought to have had a lung carcinoma, but necropsy was not performed in the last 2 instances.—E. E. S.

Tumors of the Mediastinum. FRIEDLANDER, S. O. [Cleveland, Ohio] *Ohio State M. J.* **38**:919. 1942.

A classification of all tumors, benign and malignant, occurring in the mediastinum is presented. The common symptoms and signs are listed. The author believes operation should be performed on all benign tumors, and on the malignant tumors when diagnosis can not be established otherwise. Brief case histories of patients with a teratoma, a neurogenic tumor, an aberrant pulmonary lobe, and a lymphangioendothelioma, respectively, are appended. These patients recovered following operation.—E. E. S.

Pleural Mesothelioma. KLEMPERER, P., and TEDISCHI, C. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **8**:710-720. 1942.

A case is reported of diffuse neoplasm of the pleura, arising from the mesothelial lining cells. The complex structure of the tumor, chiefly composed of lipophagic reticulum cell-like elements and of cells resembling lymphocytes in different phases of development, is explained on the basis of the multiple developmental potentiality of the mesoderm comprising the coelomic "mesothelium."—S. A. G.

Bronchial Adenoma. Inaugural Thesis. Transactions of the Minneapolis Academy of Medicine January, 1943. LOWRY, T. [Minneapolis, Minn.] *Journal-Lancet*, **63**:324-328. 1943.

Four cases are presented that were treated by local removal of the tumor by means of the bronchoscope. After giving a brief historical review of the subject, the author outlines the difficulties in diagnosis and management of this tumor.—M. E. H.

Adenoma of the Bronchus with Successful Pneumonectomy. Report of a Case. MASON, D. G., and COBERTH, T. [Univ. of Oregon Med. Sch., Portland, Ore., and The Dalles Hosp., Dalles, Ore.] *Arch. Surg.*, **45**:542-550. 1942.

A case report.—G. H. H.

Neurological Manifestations of Carcinoma of the Lung. WESSLER, H., and RABIN, C. B. [New York, N. Y.] *J. Mt. Sinai Hosp.*, **9**:850-858. 1942.

This study is based upon 300 cases of carcinoma of the lung diagnosed during life or at postmortem examination. In 62 of these cases there was clinical evidence of involvement of the nervous system. An analysis of the neurological signs and symptoms is presented in detail, and the literature is reviewed.—S. A. G.

GASTROINTESTINAL TRACT

Total Gastrectomy for Diffuse Superficial Carcinoma. BAKER, J. W. [Seattle, Washington] *Northwest Med.* **40**:277-280. 1941.

A 60 year old man in whom a roentgenogram showed a filling defect in the distal half of the stomach was operated

upon about 6 months after onset of symptoms. A radical total gastrectomy was performed including the greater omentum, duodenum, and a portion of the esophagus. The esophagus was then anastomosed to the jejunum. Postoperative complications included pneumothorax, localized atelectasis relieved by bronchoscopy, and parotitis, which cleared following roentgen and radium therapy. Whole blood, plasma, and saline were frequently given, and the patient received peptonized protein, carbohydrate, salt, and vitamin through an indwelling Levine tube. The serum protein level did not rise to a low normal level for about 1 month. The operative details are discussed.—E. E. S.

Pseudomyxoma Peritonaei in a Man. Chaffee, J. S., and LeGrand, R. H. [Hosp. of the Protestant Episcopal Church, Philadelphia, Pa.] *Arch. Surg.*, **45**:55-73. 1942.

Report of a case. The original lesion was a mucocele of the appendix. The literature is reviewed, and a discussion of the pathogenesis, clinical picture, and prognosis is given. The malignant potentialities of pseudomyxoma peritonaei arising from an ovarian cystadenoma are stressed.—G. H. H.

Surgical Problems in the Treatment of Gastric Ulcer. Colp, R. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **8**:447-453. 1942.

In a series of 165 patients with chronic gastric ulcer, operated upon at The Mount Sinai Hospital during the period from 1925 to 1935, 20% of the lesions were diagnosed roentgenologically as carcinoma of the stomach. Twelve per cent diagnosed as benign ulcer were subsequently proved to be malignant. In the past 3½ years among 28 consecutive ward cases there were 6 additional instances in which benign ulcer was suspected but in which carcinoma was disclosed at operation. The author presents one case typical of the latter group; another case of a suspected carcinoma of the stomach which on operation proved to be a chronic peptic ulcer with no evidence of tumor; and a third case in which carcinoma was suspected, and confirmed on microscopic examination of the surgical specimen.

The author's operative procedure of choice for gastric ulcer is a subtotal gastrectomy of the Bilroth II type with restoration of intestinal continuity by a Hofmeister termino-lateral gastrojejunostomy.—S. A. G.

Association of Pernicious Anemia and Carcinoma of the Stomach. Doehring, P. C., and Eusterman, G. B. [The Mayo Clinic, Rochester, Minn.] *Arch. Surg.*, **45**:554-563. 1942.

A discussion of 40 cases of associated pernicious anemia and carcinoma of the stomach. The authors believe that persons with pernicious anemia are slightly more likely than normal persons to have gastric carcinoma.—G. H. H.

Benign Tumors of the Stomach. Dudley, G. S., Miscall, L., and Morse, S. F. [New York, N. Y.] *Arch. Surg.*, **45**:702-726. 1942.

A discussion of 108 cases of benign gastric tumor. Seventy-six of the lesions were found at autopsy and 32 at operation. Nine cases are presented in detail with 24 illustrations. Many of the tumors produced no symptoms, while others caused pyloric obstruction and hemorrhage. The authors believe that the hazards of severe hemorrhage and malignant change constitute valid indications for surgical treatment of all benign gastric tumors.—G. H. H.

Early Symptoms and Signs of Cancer of the Rectum. Habermel, J. [New Albany, Ind.] *J. Indiana M. A.*, **35**:196-197. 1942.

Since syphilis of the rectum and some benign growths produce symptoms similar to those of an early carcinoma, careful examination with the aid of a proctoscope and biopsy of suspicious areas, as well as the Wassermann test and x-ray studies are urged.—E. E. S.

Carcinoid Tumor of the Appendix. Report of a Case in Which Extensive Intra-Abdominal Metastases Occurred, Including Involvement of the Right Ovary. Hopping, R. A., Dockerty, M. B., and Masson, J. C. [The Mayo Clinic, Rochester, Minn.] *Arch. Surg.*, **45**:613-622. 1942.

A case report.—G. H. H.

Resection of Carcinoma of Rectosigmoid with End-to-End Anastomosis of Sigmoid to Rectum. Lamson, O. F. [Seattle, Wash.] *Northwest Med.*, **40**:117-118. 1941.

This procedure was carried out successfully on a patient who refused permanent colostomy even though the risks include the higher mortality, difficulty in healing due to impaired blood supply, the possibility of less accuracy in the suturing of the anastomosis, and the greater likelihood of infection.—E. E. S.

Problems in Diagnosis of Cancer of the Colon. Likely, D. S. [New York, N. Y.] *Rev. Gastroenterol.*, **10**:149-153. 1943.

The importance of symptoms of change in bowel function as an early sign is stressed. More general use of the sigmoidoscope is urged. Associated conditions, such as gall bladder disease or hemorrhoids may obscure the diagnosis. All bleeding from the colon must arouse suspicion. Amebic disease may produce a tumor simulating cancer. Colonic spasm is a problem in diagnosis.—M. E. H.

Adenocarcinoma of the Sigmoid Colon, Rectum, and Anus in Children. Report of Two Cases in a 13-Year-Old Girl and an 8-Year-Old Boy with Summary of the Recorded Cases Up to 15 Years of Age. Oosting, M. [Miami Valley Hosp., Dayton, Ohio] *Ohio State M. J.*, **37**:1067-1068. 1941.

Two patients with adenocarcinoma of the rectum are reported. In the first patient, an enlarged supraclavicular node revealed the presence of carcinoma, and the primary tumor was found at necropsy. The second patient appeared perfectly healthy, but section of a rectal polyp that became prolapsed showed evidence of malignant transformation. There had been no recurrence at the time of writing.—E. E. S.

Malignant Carcinoid of the Stomach. Case Report of a Patient Treated by Subtotal Gastrectomy. Puderbach, W. J., and Ficarra, B. J. [Kings County Hosp., Brooklyn, N. Y.] *Am. J. Surg.*, **61**:121-123. 1943.

A subtotal gastrectomy was performed for this tumor that had invaded the regional lymph nodes. At present, 1 month later, the patient is living and well.—W. A. B.

The Importance of Proctoscopy in the Diagnosis and Treatment of the Lower Bowel. Reichman, H. R.

[Salt Lake City, Utah] *Rocky Mountain M. J.*, **40**:660-663. 1943.

Six cases of malignancy of the rectum were diagnosed by means of the proctoscope. In one case x-ray failed to reveal the lesion. Use of the proctoscope is an essential part of the examination of patients with rectal or colonic complaints.—M. E. H.

Treatment of Large Gastric Ulcers. Résumé of a Ten Year Study. Steigmann, F. [Cook County Hosp., and Univ. of Illinois Coll. of Med., Chicago, Ill.] *Arch. Surg.*, **45**: 764-775. 1942.

The author concludes from studies and observations during a period of 10 years on more than 200 patients with large gastric ulcers that surgical intervention is the treatment of choice. In most cases it cannot be definitely ascertained preoperatively whether the lesion is benign or malignant. Large benign ulcers tend to heal poorly, bleed dangerously, and rarely to undergo malignant change.—G. H. H.

Leukemia, Lymphosarcoma, Hodgkin's Disease

The Diagnosis of Human Leukemia. Reich, C. [New York, N. Y.] *M. Rec.*, **153**:319-321. 1941.

The mode of onset, presenting symptoms, and features of the physical examination in cases of acute and chronic leukemia are briefly described. Details of the blood smear are given. Bone marrow biopsy is often of great assistance in making the diagnosis. The differential diagnosis includes agranulocytosis, pertussis, infectious mononucleosis, pyogenic infections, tuberculosis, and the leukemoid reaction to tumors metastasizing to bone marrow.—E. E. S.

Lymphosarcoma Cutis—With a Consideration of the Clinical and Pathological Manifestations of Two Unusual Instances. Menne, F. R., and Bitar, E. [U. S. Veterans' Administration and Univ. of Oregon Med. Sch., Portland, Oreg.] *Urol. & Cutan. Rev.*, **45**:774-781. 1941.

The author discusses the previously proposed classifications of skin lymphoblastomas and points out the uncertainty in placing many lesions in one of these groups. Two cases are described in detail and illustrated. One was a 74 year old male with multiple recurring lymphosarcoma cutis. Autopsy revealed extensive internal metastases. The other was a 9 months old male infant with a single subcutaneous lymphoma. Postmortem examination revealed no internal metastases but the right cervical region was extensively involved. The lesions in both instances were very radiosensitive, but new crops of nodules would appear rapidly. The author cites the following criteria as characteristic of lymphosarcoma cutis: (1) local lymph node invasion; (2) skin invasion without ulceration, papules, exfoliation, or hemorrhagic dermatitis; (3) no pronounced changes in blood picture except myelophthisic anemia; (4) mature and malignant lymphocytes in the biopsy; (5) radiosensitivity; (6) generalized nodular spread into internal organs. He considers lymphosarcoma cutis actually to be secondary to initial involvement of internal or contiguous lymph nodes.—V. F. M.

Pituitary

Adamantinoma of the Hypophyseal Duct. MacCallum, W. G. [Johns Hopkins Med. Sch., Baltimore, Md.] *J. Mt. Sinai Hosp.*, **8**:798-804. 1942.

In the records of the Johns Hopkins Hospital there are 113 cases of tumor of the craniopharyngeal duct, arising from the cells that later form the pars intermedia and the pars tuberalis of the hypophysis. In this group the author found 12 cases of adamantinoma. He reports one in detail and summarizes 11 briefly. Photomicrographs demonstrate the characteristic structure of adamantinoma.—S. A. G.

Thyroid

Early Carcinoma in the Hyperplastic Thyroid. Emmett, J. M., and Dreyfuss, M. L. [Chesapeake and Ohio Hosp., Clifton Forge, Va.] *Arch. Surg.*, **45**:316-322. 1942.

A case report.—G. H. H.

Statistics

Annual Report of the Chief Inspector of Factories for the Year 1942. Industrial Health. Merewether, E. R. A. H. M. Stationery Office, London, England. p. 34-35.

There was a further reduction in the number of notified cases of epitheliomatous ulceration—113 (8 fatal) as compared with 128 (11 fatal) in 1941. Eighty-five (2 fatal) of these were due to pitch and tar, and 28 (6 fatal) to mineral oil. Since the beginning of the war the Registrar General has been unable to continue his usual practice of notifying fatal industrial cases that had not been notified during life; if this were possible the figures would be somewhat higher.—E. L. K.

Quarterly Return of the Registrar General. Scotland. Births, deaths, and marriages registered in the Quarter ended 31st December 1942, also preliminary return for the year 1942. Edinburgh. Published by H. M. Stationery Office, 1942.

Deaths from malignant disease in 1942 numbered 8,556 being 120 more than in 1941 and 431 above the average for the 5 years from 1937 to 1941. This is the largest number hitherto registered in Scotland but it is probable that the greater part of this increase may be attributed to the aging of the population. The death rate is 171 per 100,000 of the population estimated for 1939 (168 in 1941).—E. L. K.

Correction

The authors of "The Metabolism of 1,2-Benzanthracene in Mice and Rats" (**3**:686. 1943) point out that the last compound in Fig. 3, page 690, should be 9,10-Dimethoxy-1,2-benzanthracene, instead of 9,10-Dimethyl-1,2-benzanthacene. As this figure is a reproduction by photography of the authors' chart the fault lies in the original.